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(54) **ANTIGENIC POLYPEPTIDE OF CHLAMYDIA PNEUMONIAE**

(57) An antigenic polypeptide of *Chlamydia pneumoniae* comprising the polypeptide A containing the sequence of at least five consecutive amino acid residues in the polypeptide of SEQ ID NO: 1; a DNA coding for the polypeptide; a recombinant vector containing the DNA; a transformant containing the vector; a process for producing an anti-*C. pneumoniae* antibody by using the antigenic polypeptide as the antigen; methods for detecting and assaying the anti-*C. pneumoniae* antibody; the use of the antigenic polypeptide; a fused protein consisting of a dihydrofolate reductase and an antigenic polypeptide *C. pneumoniae*, wherein the polypeptide of SEQ ID NO: 14 has bound to the polypeptide A containing the sequence of at least five consecutive amino acid residues in the polypeptide of SEQ ID NO: 1; a DNA coding for the fused protein; a recombinant vector containing the DNA; a transformant containing the vector; a process for producing an anti-*C. pneumoniae* antibody by using the fused protein as the antigen; methods for detecting and assaying the anti-*C. pneumoniae* antibody by using the fused protein as the antigen; the use of the fused protein; a probe and a primer for detecting and assaying *C. pneumoniae* genes; methods for detecting and assaying *C. pneumoniae* genes by using the probe or primer; and the use of the probe or primer.

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Description

FIELD OF THE INVENTION

The invention relates to Chlamydia pneumoniae antigenic polypeptides, fused proteins containing the polypeptides, DNAs coding therefor, recombinant vectors carrying the DNAs, transformants containing the recombinant vectors, a method for production of antibody, a method and reagents for detection and/or measurement of antibody, a method and agents for diagnosis of Chlamydia pneumoniae infections, probes and primers for detection and/or measurement of Chlamydia pneumoniae gene, and a method and reagents for detection and/or measurement of Chlamydia pneumoniae gene. The invention can be effectively used in the pharmaceutical industry, particularly in the preparation of agents for diagnosis of Chlamydia pneumoniae infections.

BACKGROUND ART

Several kinds of species are known in Chlamydia, that is, Chlamydia trachomatis, Chlamydia psittaci, Chlamydia pecorum, Chlamydia pneumoniae and the like. Chlamydia trachomatis causes trachoma, venereal lymphogranuloma, urogenital infections, inclusion conjunctivitis, neonatal pneumonia and the like. Chlamydia psittaci causes psittacosis and the like. Chlamydia pneumoniae causes respiratory infections, atypical pneumonia and the like.

Since the symptoms of infections in the respiratory apparatus which are caused by Chlamydia pneumoniae are similar to those of infections caused by Mycoplasma pneumoniae or Influenza virus, physicians often make a wrong diagnosis. Hence, there is a need for the development of a simple method for diagnosing the infections caused by Chlamydia pneumoniae.

In general, an infection can reliably be diagnosed by detecting the causative bacterium in the infected site or by detecting an antibody against the causative bacterium in body fluids such as a sera and the like. The former method is called an antigen test and the latter is called an antibody test. Both of them are clinically important. As for Chlamydia pneumoniae, there is known an antibody test which is carried out by a method in which an antibody is detected by using an elementary body of Chlamydia pneumoniae.

However, this method has the disadvantage that the elementary body of Chlamydia pneumoniae reacts not only with an antibody against Chlamydia pneumoniae but also with antibodies against other species of Chlamydia, thus being fairly unspecific. This is because the elementary body of Chlamydia pneumoniae contains an antigen which is also present in other species of genus Chlamydia than Chlamydia pneumoniae, that is, Chlamydia trachomatis and Chlamydia psittaci.

As a plasmid which can be used for the expression of a large amount of a protein in E. coli, pBBK10MM is known (Japanese Unexamined Patent Publication No. Hei 4-117284). This plasmid can be used for the expression of a fused protein of an anti-allergic peptide with DHFR. The expressed fused protein also maintains the enzymatic activity of DHFR and can therefore be purified easily by utilizing the characteristic properties and activities of DHFR.

Genetic screening has been carried out to diagnose infections. In this screening, the presence of the gene of a microorganism to be detected in a sample is examined using nucleic acid probes and the like.

As for Chlamydia pneumoniae, there is known a genetic screening method which is carried out as disclosed in Japanese Unexamined Patent Publication No. Sho 64-500083, U.S.P. No. 5,281,518 and WO94/04549.

However, Japanese Unexamined Patent Publication No. Sho 64-500083 and U.S.P. No. 5,281,518 only disclose that a chromosomal DNA of Chlamydia pneumoniae or a DNA fragment which is obtained by cleaving the chromosomal DNA with a restriction enzyme or the like is used as a probe. The base sequences of these DNA molecules are not determined and the specificity of these probes are therefore unclear. In addition, it is difficult to determine the reaction conditions.

Although WO94/04549 discloses a method using a probe which is hybridized to ribosome RNA or DNA corresponding thereto, the specificity of these probes is not reliable because the homology of ribosomal RNA is relatively high in all organisms.

DISCLOSURE OF THE INVENTION

It is an object of the invention to provide antigenic polypeptides that do not react with antibodies against species of genus Chlamydia other than Chlamydia pneumoniae, such as Chlamydia trachomatis, Chlamydia psittaci and the like and which react only with a Chlamydia pneumoniae-specific antibody and can thereby detect the Chlamydia pneumoniae-specific antibody.

Another object of the invention is to provide a method for synthesizing large amounts of the antigenic polypeptides by using gene recombination techniques.

A further object of the invention is to provide a method for production of an anti-Chlamydia pneumoniae-specific antibody, a method and reagents for detection and/or measurement of the anti-Chlamydia pneumoniae-specific anti-

body, and agents for diagnosis of Chlamydia pneumoniae infections, all by using said antigenic polypeptides.

A still further object of the invention is to provide probes and primers for detecting and/or measuring specifically Chlamydia pneumoniae gene, a method and reagents for detection and/or measurement of Chlamydia pneumoniae gene and agents for diagnosis of Chlamydia pneumoniae infections, all by using the probes or primers.

5 An even further object of the invention is to provide antigenic polypeptides for detection of an antibody which reacts with genus Chlamydia including Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydia psittaci and the like.

SUMMARY OF THE INVENTION

10 The subject matters of the invention are as follows:

- (1) A Chlamydia pneumoniae antigenic polypeptide, which comprises polypeptide containing a sequence of at least 5 consecutive amino acids in the polypeptide of SEQ ID NO: 1 (hereinafter referred to as "polypeptide A").
- (2) The antigenic polypeptide of (1), wherein said polypeptide A is a polypeptide in which at least one amino acid is deleted from the polypeptide of SEQ ID NO: 1.
- 15 (3) The antigenic polypeptide of (1), wherein said polypeptide A is a polypeptide in which at least one amino acid in the polypeptide of SEQ ID NO: 1 is replaced with other amino acid or a polypeptide in which at least one amino acid is added in the polypeptide of SEQ ID NO: 1.
- (4) The antigenic polypeptide of (1), wherein said polypeptide A is a polypeptide in which an amino acid or a peptide sequence is bound to a sequence of at least 5 consecutive amino acids in the polypeptide of SEQ ID NO: 1.
- 20 (5) The antigenic polypeptide of (1), wherein said polypeptide A is a polypeptide containing the amino acid sequence of SEQ ID NO: 1.
- (6) The antigenic polypeptide of (1), wherein said polypeptide A is a polypeptide containing the amino acid sequence of SEQ ID NO: 2.
- 25 (7) The antigenic polypeptide of (1), wherein said polypeptide A is a polypeptide containing the amino acid sequence of SEQ ID NO: 5.
- (8) A DNA encoding the antigenic polypeptide of any one of (1)-(7), or a DNA complementary thereto.
- (9) The DNA of (8), which contains the base sequence of SEQ ID NO: 3.
- (10) The DNA of (8), which contains the base sequence of SEQ ID NO: 4.
- 30 (11) The DNA of (8), which contains the base sequence of SEQ ID NO: 7.
- (12) A recombinant vector carrying the DNA of any one of (8)-(11).
- (13) The recombinant vector of (12), which is plasmid pCPN533 α containing the base sequence of SEQ ID NO: 10.
- (14) A transformant containing the recombinant vector of (12) or (13).
- (15) A method for production of an anti-Chlamydia pneumoniae antibody,
- 35 wherein the antigenic polypeptide of any one of (1)-(7) is used as an antigen.
- (16) A method for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, wherein the antigenic polypeptide of any one of (1)-(7) is used as an antigen.
- (17) A reagent for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, which comprises the antigenic polypeptide of any one of (1)-(7) as an antigen.
- 40 (18) A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the antigenic polypeptide of any one of (1)-(7) as an active ingredient.
- (19) A fused protein of a Chlamydia pneumoniae antigenic polypeptide with dihydrofolate reductase, in which polypeptide containing a sequence of at least 5 consecutive amino acids in the polypeptide of SEQ ID NO: 1 is bound to the polypeptide of SEQ ID NO: 14 (hereinafter referred to as "polypeptide B") either directly or via an
- 45 intervening amino acid or amino acid sequence.
- (20) The fused protein of (19), wherein said polypeptide B is a polypeptide in which at least one amino acid is deleted from the polypeptide of SEQ ID NO: 1.
- (21) The fused protein of (19), wherein said polypeptide B is a polypeptide in which at least one amino acid in the polypeptide of SEQ ID NO: 1 is replaced with other amino acids or a polypeptide in which at least one amino acid is added in the polypeptide of SEQ ID NO: 1.
- 50 (22) The fused protein of (19), which is a polypeptide containing the amino acid sequence of SEQ ID NO: 15.
- (23) The fused protein of (19), which is a polypeptide containing the amino acid sequence of SEQ ID NO: 16.
- (24) A DNA encoding the fused protein of any one of (19)-(23), or a DNA complementary thereto.
- (25) The DNA of (24), which contains the base sequence of SEQ ID NO: 17.
- 55 (26) The DNA of (24), which contains the base sequence of SEQ ID NO: 18.
- (27) A recombinant vector carrying the DNA of any one of (24)-(26).
- (28) The recombinant vector of (27), which is plasmid pCPN533T.
- (29) A transformant containing the recombinant vector of (27) or (28).
- (30) A method for production of an anti-Chlamydia pneumoniae antibody, wherein the fused protein of any one of

(19)-(23) is used as an antigen.

(31) A method for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, wherein the fused protein of any one of (19)-(23) is used as an antigen.

(32) A reagent for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, which comprises the fused protein of any one of (19)-(23) as an antigen.

(33) A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the fused protein of any one of (19)-(23) as an active ingredient.

(34) A probe for detection and/or measurement of Chlamydia pneumoniae gene, which comprises any one of

- (a) a DNA containing a sequence of at least 10 consecutive bases in the DNA of SEQ ID NO: 3,
- (b) a DNA complementary to DNA (a), or
- (c) a DNA having at least 90% homology to DNA (a) or (b).

(35) The probe of (34), which contains the base sequence of SEQ ID NO: 19.

(36) The probe of (34), which contains the base sequence of SEQ ID NO: 20.

(37) A method for detection and/or measurement of Chlamydia pneumoniae gene, characterized in that the probe of any one of (34)-(36) is used.

(38) A reagent for detection and/or measurement of Chlamydia pneumoniae gene, which comprises the probe of any one of (34)-(36).

(39) An agent for diagnosis of a Chlamydia pneumoniae infection, which comprises the probe of any one of (34)-(36) as an active ingredient.

(40) A primer for detection and/or measurement of Chlamydia pneumoniae gene, which comprises any one of

- (a) a DNA containing a sequence of at least 10 consecutive bases in the DNA of SEQ ID NO: 3,
- (b) a DNA complementary to DNA (a), or
- (c) a DNA having at least 90% homology to DNA (a) or (b).

(41) The primer of (40), which contains the base sequence of SEQ ID NO: 19.

(42) The primer of (40), which contains the base sequence of SEQ ID NO: 20.

(43) A method for detection and/or measurement of Chlamydia pneumoniae gene, wherein the primer of any one of (40)-(42) is used.

(44) A reagent for detection and/or measurement of Chlamydia pneumoniae gene, which comprises the primer of any one of (40)-(42).

(45) A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the primer of any one of (40)-(42) as an active ingredient.

(46) A Chlamydia pneumoniae antigenic polypeptide, which is selected from the group consisting of

- (a) the polypeptide of SEQ ID NO: 5,
- (b) a polypeptide in which at least one amino acid is deleted from the polypeptide of SEQ ID NO: 5,
- (c) a polypeptide in which at least one amino acid in the polypeptide of SEQ ID NO: 5 is replaced with another amino acid, and
- (d) a fused polypeptide of any one of (a)-(c) with another amino acid or peptide.

(47) A Chlamydia pneumoniae antigenic polypeptide, which is selected from the group consisting of

- (a) the polypeptide of SEQ ID NO: 6,
- (b) a polypeptide in which at least one amino acid is deleted from the polypeptide of SEQ ID NO: 6,
- (c) a polypeptide in which at least one amino acid in the polypeptide of SEQ ID NO: 6 is replaced with another amino acid, and
- (d) a fused polypeptide of any one of (a)-(c) with another amino acid or peptide.

(48) A DNA encoding the polypeptide of (46), or a DNA complementary thereto.

(49) A DNA encoding the polypeptide of (47), or a DNA complementary thereto.

(50) The DNA of (48), wherein said DNA encoding the polypeptide of (46) is the DNA of SEQ ID NO: 7.

(51) The DNA of (49), wherein said DNA encoding the polypeptide of (47) is the DNA of SEQ ID NO: 8.

(52) A recombinant vector carrying the DNA of any one of (48)-(51).

DETAILED DESCRIPTION OF THE INVENTION

In the specification, deoxynucleotides having only one base are referred to as "monodeoxynucleotides" and deoxynucleotides having at least two bases are referred to as "DNAs", unless otherwise indicated.

5 The invention will now be explained in detail.

Antigen polypeptide

The antigen polypeptide of the present invention is formed of polypeptides containing at least five continued amino acid sequences in a polypeptide of SEQ ID No. 1 (hereinafter referred to as "Polypeptide A") from the viewpoint of the minimum size in which a peptide is allowed to possess antigenicity.

Since the antigen-antibody reaction can be expected to gain in sensitivity in proportion as the length of amino acid sequence increases, the polypeptide A is appropriately formed of not less than 20, preferably not less than 100, and more preferably not less than 250 amino acids.

15 So long as the polypeptide A possesses the antigenicity inherent in *Chlamydia pneumoniae*, it tolerates the loss of amino acids (1 - 250 amino acids, for example) from the polypeptide of SEQ ID No. 1. If the number of missing amino acids is unduly large, the polypeptide A will tend to suffer the antigenicity inherent in *Chlamydia pneumoniae* to be impaired.

When the number of missing amino acids is large (five or more, for example), the polypeptide A prefers such missing amino acids (five or more, for example) to occur in a continued series for the sake of retaining the antigenicity of *Chlamydia pneumoniae*.

So long as the polypeptide A possesses the antigenicity inherent in *Chlamydia pneumoniae*, it tolerates the substitution of part of the amino acids (1 - 100 amino acids, for example) by other amino acids or the insertion of amino acids (1 - 100 amino acids, for example) in the polypeptide of SEQ ID No. 1. If the number of amino acids involved in the substitution or insertion is unduly large, the polypeptide A will tend to suffer the antigenicity inherent in *Chlamydia pneumoniae* to be impaired. When the number of amino acids involved in the substitution or insertion is large (five or more, for example), the polypeptide A prefers the amino acids (five or more, for example) to occur in a continued series for the sake of retaining the antigenicity of *Chlamydia pneumoniae*. The amino acids to be involved in the substitution are preferred to possess such similar qualities as are observed in the substitution between glycine and alanine, for example.

30 So long as the polypeptide A possesses the antigenicity inherent in *Chlamydia pneumoniae*, it may be a polypeptide having amino acids or peptides ligated directly or through the medium of an intervening amino acid sequence to at least five continued amino acid sequences in the polypeptide of SEQ ID No. 1.

The peptides for the ligation are appropriately formed of not more than 1000 amino acid sequences, preferably not more than 500 amino acid sequences, and more preferably not more than 200 amino acid sequences for the sake of retaining the antigenicity inherent in *Chlamydia pneumoniae*.

35 As concrete examples of such amino acids or peptides, leucine, leucine-methionine, dihydrofolic acid reductase (DHFR), and β -galactosidase may be cited.

As concrete examples of the polypeptide A using DHFR or β -galactosidase as a peptide, DHFR-*Chlamydia pneumoniae* antigen polypeptide-fused protein and β -galactosidase-*Chlamydia pneumoniae* antigen polypeptide-fused protein may be cited. DHFR or β -galactosidase may be ligated either directly or through the medium of an intervening amino acid sequence with *Chlamydia pneumoniae* antigen polypeptide.

40 As concrete examples of the polypeptide A, the polypeptides of SEQ ID No. 1, SEQ ID No. 2, and Sequence No. 5 may be cited.

Though the intervening amino acid sequence is not defined particularly, the amino acid sequences of leucine and leucine-methionine are examples.

45 As concrete examples of the fused protein of the present invention, the polypeptide formed of amino acid sequences of SEQ ID No. 15 and the polypeptide formed of amino acid sequences of SEQ ID No. 16 may be cited.

Among the fused proteins cited above, the polypeptide formed of the amino acid sequences of SEQ ID No. 15 including the whole antigen polypeptide of 53 kDa of *Chlamydia pneumoniae* proves particularly advantageous.

50 The method of chemical synthesis and the method of gene recombination are available for the production of the antigen polypeptide of this invention.

The polypeptide of SEQ ID No. 1 of this invention is an antigen polypeptide formed of 488 amino acid residues as shown in the table of sequences.

The polypeptide of SEQ ID No. 2 of this invention is an antigen polypeptide formed of 271 amino acid residues as shown in the table of sequences.

55 The polypeptide of SEQ ID No. 5 of this invention is an antigen polypeptide formed of 259 amino acid residues as shown in the table of sequences.

Among other antigen polypeptides mentioned above, the polypeptide of SEQ ID No. 1 containing the whole antigen polypeptide of 53 kDa of *Chlamydia pneumoniae* proves particularly advantageous.

Method for production of antigen polypeptide

The method of chemical synthesis and the method of gene recombination are available for the production of the antigen polypeptide of this invention.

Among the methods of chemical synthesis is counted the MAP (multiple antigen peptide) method, for example. The MAP method befits the synthesis of a peptide formed of not more than 30 amino acid sequences. This synthesis can be implemented by the use of a commercially available peptide synthesizing device.

Among the methods of gene recombination is counted a method which comprises inserting a DNA coding for the antigen polypeptide of this invention in a vector thereby constructing a recombinant vector, inserting the recombinant vector in a host thereby producing a transformant, and isolating the peptide aimed at from the transformant.

The DNA coding for the antigen polypeptide of this invention will be described afterward.

The vector may be plasmid, phage, etc.

As concrete examples of the host, *Escherichia coli*, *Bacillus subtilis*, yeast, etc. may be cited.

Now, the method for forming the transformant and the method for refining the peptide aimed at by the use of the transformant will be described in detail below.

Preparation of Recombinant Vector Carrying the DNA Encoding the Antigenic Polypeptide and Transformants Containing the Same

The λ phage obtained by screening (see *infra*) is already a kind of recombinant vector carrying the DNA of the invention. Additional recombinant vectors can be prepared by inserting in a known plasmid vector or phage vector the DNA encoding the *Chlamydia pneumoniae* antigenic polypeptide (see *infra*) in a conventional procedure. In this case, a linker may be used if necessary. As the known plasmid vector, pBR322, pUC18, pUC19, pBBK10MM or the like can be used. Plasmids pBR322, pUC18 and pUC19 are commercially available and pBBK10MM is described in detail in Japanese Unexamined Patent Publication No. Hei 4-117284. As the phage vector, λ gt11 phage, λ gt10 phage or the like can be used. In any case, recombinant vectors corresponding to the parent vectors used can be obtained.

The recombinant vectors carrying the DNA of the invention include plasmid pCPN533 α , 53-3S λ phage and the like (see *infra*).

The obtained recombinant vector is introduced into a host to prepare a transformant. If an *E. coli*-derived plasmid or λ phage is used, an *E. coli* strain such as HB 101 can be used as a host. The host is treated to become a competent cell. A competent cell obtained by treating *E. coli* strain HB101 is commercially available from Takara Shuzo Co., Ltd. A method of introducing the recombinant vector into a host to prepare a transformant is described in "Molecular Cloning".

The obtained transformant is cultured to form colonies. Plasmid DNAs are obtained from each of the colonies and cleaved with an appropriate restriction enzyme. A transformant having a desired recombinant plasmid is selected according to the results of agarose gel electrophoretic analysis of the cleaved plasmid DNA. The plasmid vectors thus prepared include plasmid pCPN533 α .

Examples of the transformant thus prepared include *E. coli* strain HB101 containing the recombinant vector pCPN533 α .

Preparation of Recombinant Vectors Carrying the DNA Encoding Fused Protein of the *Chlamydia pneumoniae* Antigenic Polypeptide with DHFR and Transformants Containing the Same

The DNA molecule encoding the *Chlamydia pneumoniae* antigenic polypeptide (see *infra*) is ligated to the DNA molecule encoding DHFR (see *infra*) by means of a commercially available kit. In the ligation, a linker may be used if necessary. A DNA ligation kit (Takara Shuzo Co., Ltd) can be used as a commercially available kit. If the DNA obtained by the ligation does not have a replication origin and does not therefore function as a plasmid, the DNA is inserted in a separate plasmid vector, which may be pBR322, pUC18 or the like.

The ligated DNA is introduced into a host to prepare a transformant. If an *E. coli*-derived plasmid is used, an *E. coli* strain such as HB 101 can be used as a host. The host is treated to become a competent cell. A competent cell obtained by treating *E. coli* strain HB101 is commercially available from Takara Shuzo Co., Ltd. The method of introducing the ligated DNA into a host to prepare a transformant is described in "Molecular Cloning".

The obtained transformant is cultured to form colonies. Plasmid DNAs are obtained from each of the colonies and cleaved with an appropriate restriction enzyme. A transformant having a desired recombinant plasmid is selected according to the results of agarose gel electrophoretic analysis. An example of the plasmid vector thus prepared is plasmid pCPN533T.

An example of the transformant thus prepared is *E. coli* strain HB101 containing the recombinant vector pCPN533T.

The transformant is cultured by shaking an incubator containing the transformant at an appropriate temperature in a medium that allows the transformant to grow until a sufficient amount of the desired antigenic polypeptide is accumu-

lated in the transformant. If *E. coli* strain HB101 containing the recombinant vectors pCPN533 α or pCPN533T are used as a transformant, the cell is cultured while shaking in ampicillin-containing LB medium at 37 °C overnight. Subsequently, the culture is inoculated in ampicillin-containing TB medium and further cultured while shaking at 37°C overnight. A method for preparing the TB medium is described in "Molecular Cloning".

- 5 The cultured transformant is harvested by centrifugation and suspended in a buffer. The transformant is disrupted by sonication of the suspension. If the transformant is *E. coli*, the cell may be lysed by successively adding lysozyme and an SDS-containing buffer to the suspension.

When the polypeptide aimed at is secretory in quality, the culture broth is centrifuged to obtain the supernatant.

- After the disruption of the transformant, the cell residue is removed by centrifugation, thereby obtaining the supernatant. Streptomycin sulfate is added to the supernatant. The mixture is stirred for a certain period of time and centrifuged to precipitate nucleic acids, thereby obtaining the supernatant.

This supernatant is precipitated with ammonium sulfate and centrifuged. Generally, the precipitate is recovered as the product. Since the supernatant possibly contains the peptide aimed at, the practice of sampling and analyzing the supernatant thereby confirming the presence or absence of the peptide proves advantageous.

- 15 Either the solution of the precipitate in a small amount of buffer solution or the supernatant is fractionated by liquid chromatography. The proteins contained in the fractions are blotted by the Western blotting method using a *Chlamydia pneumoniae*-specific monoclonal antibody to obtain the fractions containing antigen polypeptide. When the polypeptide A is a protein fused with DHFR, a Methotrexate column can be used as the column for the liquid chromatography. Specific procedures of the removal of residues such as a cell membrane and the like, the removal of DNA by addition of streptomycin sulfate, the recovery of proteins by addition of ammonium sulfate and a Western blotting method are described in "Molecular Cloning".

DNAs Encoding the Antigenic Polypeptides

- 25 In the invention, the DNA encoding the polypeptide of SEQ ID NO: 1 means DNAs selected from the group of DNAs which are obtained by translating the amino acids of the polypeptide of SEQ ID NO: 1 to triplets in accordance with the genetic code (each amino acid is assigned 1-6 sets of nucleotide sequences). This group of DNAs includes the DNA of SEQ ID NO: 3.

- The DNA encoding the antigenic polypeptide A means DNAs encoding the polypeptide A. These DNAs are selected from the group of DNAs which are obtained by translating the amino acid sequence for the polypeptide A to triplets in accordance with the genetic code.

As the polypeptide A, those polypeptides which have been described under the item "Antigenic Polypeptides" above may be given. As the DNA encoding the polypeptide A, nucleotide sequences which correspond to the amino acid sequences for those polypeptides may be given.

- 35 Similarly, the DNA encoding the polypeptide of SEQ ID NO: 2 means DNAs selected from the group of DNAs which are obtained by translating the amino acids of the polypeptide of SEQ ID NO: 2 to triplets in accordance with the genetic code. This group of DNAs includes the DNA of SEQ ID NO: 4.

- Additionally, the DNA encoding the polypeptide of SEQ ID NO: 5 means DNAs selected from the group of DNAs which are obtained by translating the amino acids of the polypeptide of SEQ ID NO: 5 to triplets in accordance with the genetic code. This group of DNAs includes the DNA of SEQ ID NO: 7.

Moreover, the DNA encoding the polypeptide of SEQ ID NO: 6 means DNAs selected from the group of DNAs which are obtained by translating the amino acids of the polypeptide of SEQ ID NO: 6 to triplets in accordance with the genetic code. This group of DNAs includes the DNA of SEQ ID NO: 8.

- 45 DNAs encoding the fused proteins comprise codons corresponding to the amino acid sequence of the fused protein. The DNAs include but are not limited to the DNAs of SEQ ID NOs: 17 and 18.

The base sequence of SEQ ID No. 17 is the base sequence of the DNA coding for the fused protein of DHFR and the whole antigen polypeptide of 53 kDa of *Chlamydia pneumoniae* and the base sequence of SEQ ID No. 18 is the base sequence of the DNA coding for the fused protein of DHFR and (part of) the antigen polypeptide of 53 kDa of *Chlamydia pneumoniae*.

- 50 These DNA's can be manufactured by the method of chemical synthesis or the method of gene recombination.

Among the methods of chemical synthesis is counted the phosphoramidite method which fits the synthesis of a DNA formed in a length of not more than 100 base sequences. This chemical synthesis can be attained by a commercially available DNA synthesizing device.

- Among the methods of gene recombination are counted a method for cloning the DNA from the elementary body of *Chlamydia pneumoniae* in the manner already described and the PCR method utilizing the already acquired DNA as a template and using a primer manufactured by adopting the base sequence at a position arbitrarily selected in that DNA. The method of gene recombination is capable of manufacturing a long DNA of more than 100 bases.

Now, the method for cloning the DNA coding for the antigen polypeptide from the elementary body of *Chlamydia pneumoniae* will be described in detail below.

Culture of Chlamydia pneumoniae

A suspension of cells is prepared from cultured HL cells. The supernatant of the culture is removed and the suspension of Chlamydia pneumoniae is then added to the resulting cell sheet. After incubation, Chlamydia pneumoniae-infected HL cells are obtained by centrifugation. As Chlamydia pneumoniae, strain YK41 (Y. Kanamoto et al., Microbiol. Immunol., Vol. 37, p.495-498, 1993) can be used.

Purification of Elementary Body of Chlamydia pneumoniae

The Chlamydia pneumoniae-infected HL cells are disrupted and centrifuged, thereby recovering the supernatant. The obtained supernatant is layered onto a continuous density gradient solution containing Urografin (Schering) is centrifuged.

The yellowish white band was recovered because in the preliminary experiment, it was confirmed to contain the elementary body of Chlamydia pneumoniae with the aid of an electron microscope.

Preparation of Genomic DNA of Chlamydia pneumoniae

The elementary body of Chlamydia pneumoniae is suspended in 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM ethylenediaminetetraacetate (EDTA) (hereinafter referred to as "TE buffer"). To the resulting suspension are added a 1% aqueous solution of sodium dodecyl sulfate (SDS) and an aqueous solution of Proteinase K (1 mg/ml) and the elementary body is lysed while incubating. To the resulting solution is added phenol saturated with 0.1 M Tris-HCl buffer (pH 8.0). The mixture is stirred and centrifuged to recover an aqueous layer. The obtained aqueous layer is treated successively with RNase and phenol/chloroform/isoamyl alcohol, followed by ethanol precipitation. As a result, genomic DNA of Chlamydia pneumoniae is obtained.

Preparation of Genomic DNA Expression Library

The genomic DNA is digested with restriction enzymes *AccI*, *HaeIII* and *AluI*. The digest is treated with phenol/chloroform/isoamyl alcohol and subjected to ethanol precipitation to yield partially digested DNAs. To the partially digested DNAs are added a linker, adenosine 5'-triphosphate (hereinafter abbreviated to "ATP") and T4 ligase, thereby ligating the linker to the partially digested DNAs.

The linker-ligated partially digested DNAs are applied to a Chroma spin 6000 column in which the mobile phase is 10 mM Tris-HCl buffer containing 0.1 M NaCl and 1 mM EDTA. The eluate is collected and fractions containing 1-7 kbp DNA fragments are recovered. To the resulting fractions are added ATP and T4 polynucleotide kinase and a reaction is conducted to phosphorylate the 5' end of the DNA fragments. The reaction solution is treated with phenol/chloroform/isoamyl alcohol and subjected to ethanol precipitation to yield 5'-end-phosphorylated DNA fragments.

To the resulting DNA fragments are added λ gt11 DNA preliminarily digested with restriction enzyme *EcoRI*, ATP and T4 ligase and a reaction is conducted. The resulting recombinant λ gt11 DNA is packaged with a commercially available packaging kit to prepare a genomic DNA expression library.

Cloning of DNA Encoding Antigenic Polypeptide

Cultured cells of *E. coli* strain Y1090r are infected with the genomic DNA expression library and incubated in an agar medium. A protein produced in the cells by the expression of the inserted DNA is transferred to a nitrocellulose filter immersed in an aqueous solution of isopropylthio- β -D-galactoside (IPTG). The filter is blocked with a bovine serum albumin and washed. The filter is then reacted with a Chlamydia pneumoniae-specific monoclonal antibody. As the Chlamydia pneumoniae-specific monoclonal antibody, AY6E2E8 and SCP53 can be used. A hybridoma cell line forming AY6E2E8 has been deposited with the National Institute of Bioscience and Human-Technology, the Agency of Industrial Science and Technology (1-3, Higashi 1 chome Tsukubashi Ibaraki-ken 305, Japan) as FERM BP-5154 under the terms of the Budapest Treaty. A hybridoma cell line forming SCP53 is disclosed in J. Clin. Microbiol., Vol.132, p.583-588, 1994. After the reaction, the filter is washed and reacted with an anti-mouse IgG antibody labeled with an enzyme such as peroxidase or the like. After the reaction, the filter is washed and reacted with a color-developing substrate solution. As the color-developing substrate solution, a mixture of an aqueous solution of hydrogen peroxide and a solution of 4-chloro-1-naphthol in methanol can be used. After the reaction, the filter is washed and dried in air.

Plaques corresponding to the color-developing spots on the filter are identified and λ phage contained in the plaques is obtained. The above procedure is repeated until all the plaques react with the aforementioned monoclonal antibody. As a result, the DNA encoding an antigenic polypeptide is cloned and λ phage expressing the Chlamydia pneumoniae-specific antigenic polypeptide having reactivity with the Chlamydia pneumoniae-specific monoclonal antibody is obtained.

Production of DNA Encoding the Chlamydia pneumoniae-Specific Antigenic Polypeptide

E. coli strain Y1090r- is infected with the obtained λ phage and cultured to yield a large amount of λ phage. DNA molecules are obtained and purified from the λ phage using a commercially available kit. To the obtained DNA molecules are added a primer, Taq polymerase and deoxynucleotides. The steps of heating, cooling and incubating are repeated, thereby amplifying the DNA molecule inserted in λ gt11. λ gt11 forward primer and λ gt11 reverse primer (Takara Shuzo Co. Ltd.) can be used as primers and AmpliTaq DNA polymerase can be used as a Taq polymerase. A general procedure of DNA amplification is known as the PCR method, which is described in detail in J. Sambrook et al., Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press (1989) (hereinafter referred to as "Molecular Cloning").

The amplified DNA is obtained and its base sequence is determined and analyzed. The amplified DNA can be obtained with a commercially available kit such as Wizard PCR Prep kit (Promega). The base sequence can be determined by fluorescence-labeled terminator cycle sequencing using Taq polymerase. This sequencing can be performed with a kit commercially available from Perkin-Elmer Japan. For analysis of the base sequence, a commercially available apparatus such as Model 373A DNA Sequencer (Applied Biosystems) can be used.

Following the determination of the base sequence, the base sequence of the DNA is analyzed using a DNA sequencing software package such as DNASIS (Hitachi Software Engineering) to estimate an editing, junctional and amino acid-translational regions.

If it is found that a full-length gene has not been obtained, DNA molecules upstream and downstream of the available DNA are obtained by genome walking. The genome walking can be performed with a kit commercially available from Takara Shuzo Co., Ltd.

Preparation of DNA Encoding DHFR

DNA encoding DHFR is obtained by digesting the DNA with a restriction enzyme from a plasmid vector containing the DNA or by amplifying the DNA by PCR using a template plasmid DNA or genomic DNA containing the DNA with an appropriate primer.

In the former method, plasmid vector pBBK10MM and recombinant vector pCPN533T of the invention can be used as the plasmid vector containing DNA encoding DHFR. E. coli containing pCPN533T and E. coli containing pBBK10MM have been deposited with the National Institute of Bioscience and Human-Technology, the Agency of Industrial Science and Technology as FERM BP-5222 and FERM BP-2394, respectively. Plasmid pCPN533T can be obtained from the deposited E. coli by a conventional method for obtaining plasmid DNA, which is described in "Molecular Cloning". When plasmid pBBK10MM is used, a DNA fragment having a length of about 4.8 kbp may be excised with restriction enzymes BamHI and XhoI.

In the latter method, pBBK10MM and pCPN533T (see supra) can be used as a plasmid DNA and genomic DNA of Bacillus subtilis can be used as a genomic DNA. Genomic DNA can be obtained by a conventional method for obtaining genomic DNA, which is described in "Molecular Cloning".

The primer to be used in the latter method can be designed and synthesized in consideration of base sequences at the 5' and 3' ends of DNA encoding DHFR. For example, an oligonucleotide having the 1-20 sequence in the base sequence of SEQ ID NO: 17 and one having a sequence complementary to the 461-480 sequence in the base sequence of SEQ ID NO: 5 can be used. These oligonucleotides can be synthesized chemically with a commercially available DNA synthesizer.

In the antigen polypeptides mentioned above, the polypeptide of SEQ ID NO. 1 containing the whole antigen polypeptide of 53 kDa of Chlamydia pneumoniae is particularly preferred.

Method of Production of Anti-Chlamydia pneumoniae Antibody by Using the Antigenic Polypeptide as Antigen

An anti-Chlamydia pneumoniae antibody can be produced by immunizing a mouse with the antigenic polypeptide of the invention as an antigen, separating a spleen cell from the immunized mouse, fusing the spleen cell with a myeloma cell line to produce hybridomas, selecting a hybridoma recognizing the Chlamydia pneumoniae 53 kDa antigenic polypeptide from the produced hybridomas and culturing the selected hybridoma.

Exemplary myeloma cell lines include P3X63Ag8.653 (ATCC CRL-1580) and P3/NSI/1-Ag4-1 (ATCC TIB-18).

The anti-Chlamydia pneumoniae antibody is produced by a known general procedure for obtaining antibodies by immunization of mouse, except that the antigenic polypeptide of the invention is used as an antigen.

Method and Reagents for Detection and/or Measurement of Anti-Chlamydia pneumoniae Antibody Using the Antigenic Polypeptide as Antigen, and Agents for Diagnosis of Chlamydia pneumoniae Infections Comprising the Antigenic Polypeptide as Active Ingredient

A method for detection and/or measurement of an anti-Chlamydia pneumoniae antibody comprises, for example, the steps of immobilizing the antigenic polypeptide on a support, applying a sample, washing, adding a labeled secondary antibody, washing and detecting and/or measuring the label either directly or indirectly.

Examples of the support include latex particles, cellulose threads, plastic assay plates and particles and the like. The antigenic polypeptide may be immobilized on the support through covalent bonding or physical adsorption.

Examples of the sample include human sera and the like. It is preferred to block the surface of the support with bovine serum albumin or the like before the addition of a sample so as to insure that other antibodies in the sample will not bind to the support unspecifically.

The support is washed with a surfactant-containing phosphate buffer or the like.

An example of the labeled secondary antibody is a labeled anti-human monoclonal antibody. Useful labels include various kinds of enzymes such as alkaline phosphatase, luciferase, peroxidase, β -galactosidase and the like, various fluorescent compounds such as fluorescein and the like. A chemical compound such as biotin, avidin, streptoavidin, digoxigenin or the like may be inserted between the antibody and the label.

When the label is an enzyme, it may be detected and/or measured by adding a substrate and detecting and/or measuring the light emission or color development which occurs due to the catalytic action of the enzyme or by measuring the change in light absorbance. When the label is a fluorescent compound, it may be detected and/or measured by irradiating the reaction system with UV light and detecting and/or measuring the emitted fluorescence. A sensitizer may be used if necessary.

Reagents for detection and/or measurement of the anti-Chlamydia pneumoniae antibody using the antigenic polypeptide of interest as an antigen include the antigenic polypeptides which are immobilized on a support and those with which the necessary amounts of the secondary antibody and the substrate are enclosed.

The aforementioned reagents can be used as agents for diagnosis of Chlamydia pneumoniae infections.

Probes and Primers for Detection and/or Measurement of Chlamydia pneumoniae Gene

DNA encoding the Chlamydia pneumoniae 53 kDa antigenic polypeptide has the base sequence of SEQ ID NO: 3. The probes and primers of the invention comprise DNA containing any one of

- (a) a DNA containing a sequence of at least 10 consecutive bases in the DNA of SEQ ID NO: 3,
- (b) a DNA complementary to DNA (a), or
- (c) a DNA having at least 90% homology to DNA (a) or (b).

The length of the base sequence of the probes and primers is preferably 10-50 bp, more preferably 15-20 bp.

Specific examples of the probes and primers of the invention include a DNA comprising the base sequence of SEQ ID NO: 19 and a DNA comprising the base sequence of SEQ ID NO: 20.

The probes and primers of the invention can be synthesized easily with a commercially available DNA synthesizer. DNA synthesizers are commercially available from Applied Biosystems and the like. Alternatively, the probes and primers of the invention can be prepared by chemically synthesizing a short DNA fragment and synthesizing a long DNA fragment by PCR using the short DNA as a primer.

The probes and primers of the invention include those prepared by labeling such DNAs.

Exemplary labels include chemical compounds such as biotin, avidin, streptoavidin, digoxigenin and the like; enzymes such as alkaline phosphatase, luciferase, peroxidase, β -galactosidase and the like; and fluorescent compounds such as fluorescein and the like. Biotin may be bound to the probes by, for example, adding biotinylated deoxyuridine 5'-triphosphate to the probes in the presence of a terminal transferase. A kit containing a terminal transferase and biotinylated deoxyuridine 5'-triphosphate can be purchased from Boehringer Mannheim. In the case where a label other than biotin is to be bound, a commercially available kit can also be used. Such a kit can be purchased from Takara Shuzo Co., Ltd and TOYOBO CO., LTD. Alternatively, the label may be bound by a method described in "Molecular Cloning".

If desired, radioactive isotopes can be used as labels. In this case, (γ - 32 P)dATP is added to the probes and primers in the presence of T4 polynucleotide kinase. A general procedure of labeling with a radioactive isotope is described in "Molecular Cloning". T4 polynucleotide kinase can be purchased from TOYOBO CO., LTD. and (γ - 32 P)dATP from Amersham.

RNAs corresponding to the base sequences of the probes and primers of the invention, that is, nucleic acids in which thymine is replaced with uracil in the base moiety and in which deoxyriboses are replaced with riboses in the sugar chain, can be used as the probes and primers of the invention instead of the aforementioned probes and primer

comprising DNAs as structural units. These probes and primers comprising RNAs as structural units can be used in the method and reagents for detection and/or measurement of the invention.

Method for Detection and/or Measurement of Chlamydia pneumoniae Gene

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Chlamydia pneumoniae gene is detected and/or measured by, for example, separating DNA in a sample on the basis of the difference in molecular weight by electrophoresis, transferring the obtained DNA to a nitrocellulose filter, nylon membrane filter or the like for its identification, adding the labeled probe of the invention, and detecting and/or measuring the label. This method is called the Southern blotting technique and its general procedure is described in "Molecular Cloning".

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Chlamydia pneumoniae gene is detected and/or measured with the primer of the invention by, for example, the PCR method which was described above. The method for detecting and/or measuring Chlamydia pneumoniae gene by PCR using the primer of the invention comprises the following steps.

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- (i) A buffer containing the primer of the invention, DNA polymerase, dATP, dCTP, dGTP and dTTP is added to a sample containing DNA and the mixture is heated.
- (ii) The reaction solution is cooled, held at a constant temperature and heated.
- (iii) Step (ii) is repeated.
- (iv) The DNA contained in the reaction solution is detected and/or measured.

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The DNA-containing sample to be used in step (i) may be nucleic acids as extracted from tunica mucosa pharyngis of a patient.

The DNA polymerase to be used in step (i) may be a Taq polymerase, which can be purchased from TOYOBO CO., LTD.

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In step (i), the mixture is heated by, for example, leaving it to stand at 90-100°C for 0.5-10 minutes.

In step (ii), the reaction solution is cooled by, for example, leaving it to stand at 45-65°C for 0.5-5 minutes, held at a constant temperature by, for example, at 70-80°C for 1-10 minutes, heated by, for example, leaving it to stand at 90-100°C for 0.5-5 minutes.

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The heating in step (i), and cooling, holding at a constant temperature and heating in step (ii) can be carried out by using a DNA thermal cycler[®] (Perkin-Elmer Cetus).

Step (iii) may be repeated any number of times, preferably about 30 times.

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The DNA contained in the reaction solution is detected and/or measured in step (iv) by, for example, electrophoresing the reaction solution with an agarose gel containing ethidium bromide, and thereby separating the DNA in the reaction solution on the basis of the difference in molecular weight and irradiating the agarose gel with UV light. If the primer of the invention is a labeled one, DNA is detected and/or measured with the aid of the label.

In another embodiment of the invention, after steps (i)-(iii), the primer of the invention may be replaced with one having another base sequence and steps (i)-(iii) are repeated, followed by step (iv).

Reagents for Detection and/or Measurement of Chlamydia pneumoniae Gene

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An exemplary reagent for detection and/or measurement of Chlamydia pneumoniae gene according to the invention is an aqueous solution of the probe or primer of the invention which is packed frozen in a plastic container.

BEST MODE FOR CARRYING OUT THE INVENTION

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Now, this invention will be described in detail below with reference to examples. It is to be distinctly understood that the invention is not limited in any sense to these examples.

Now, the component steps of the process from the culture of host cells of Chlamydia pneumoniae through the determination of gene DNA sequence/amino acid sequence of the antigenic polypeptide of Chlamydia pneumoniae will be described below in the order of their occurrence.

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Example 1: Preparation of DNA coding for 53K antigenic polypeptide specific to Chlamydia pneumoniae

(A) Culture of host cells (HL cells)

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The HL cells cultured in advance confluent on the bottom surface of a plastic culture flask (75 cm²) were washed with 5 ml of a magnesium-free (-) solution of a phosphate buffer physiological saline solution (hereinafter referred to as "PBS"), coated throughout on the entire surface thereof with 5 ml of a PBS containing 0.1% (w/v) trypsin, deprived of the excess solution, kept warmed at 37 °C for 10 minutes, and made to add 5 ml of a Dulbecco MEM culture medium

containing 10% (v/v) bovine fetal serum. The HL cells adhering to the flask interior were removed by pipetting to obtain a cell suspension.

The culture in a plastic culture flask (75 cm²) was implemented by charging the culture flask with 1 ml of the cell suspension mentioned above and 5 to 20 ml of the Dulbecco MEM culture medium containing 10% (v/v) bovine fetal serum and the culture in a 6-well plastic culture vessel was effected by placing in each of the six wells 4 ml of a mixed solution consisting of 8 ml of the cell suspension mentioned above and 292 ml of the Dulbecco MEM culture medium containing 10% bovine fetal serum and performing culture under an ambience containing 5% (v/v) carbon dioxide gas.

(B) Culture of *Chlamydia pneumoniae* YK41

From the culture solution of the HL cells propagated in a 6-well plastic culture vessel (on the bottom surface thereof), the supernatant was removed with a pipet. The residual cell sheet in the culture vessel, after adding 2 ml per well of the suspension of the YK41 strain of *Chlamydia pneumoniae* (Kanamoto et al., Microbiol. Immunol., Vol. 37, p.495-498, 1993) [the supernatant obtained by diluting a preserved solution of *Chlamydia pneumoniae* YR41 to 12 to 24 times the original volume with an aqueous solution containing 75 g of sucrose, 0.52 g of monopotassium phosphate, 1.22 g of dipotassium phosphate, and 0.72 g of glutamic acid liter (hereinafter referred to as "SPG"), treating the diluted solution with a supersonic wave for one minute, and subjecting the resultant diluted solution to centrifugal separation at 2,000 rpm for three minutes], was subjected to centrifugal adsorption at 2,000 rpm for one hour. After the centrifugal adsorption, the *Chlamydia pneumoniae* suspension was removed from the resultant cell sheet. The residual cell sheet, after adding 4 ml per well of a Dulbecco MEM culture medium containing 1 µg of cyclo-heximide per ml and 10% (v/v) of bovine fetal serum, was cultured at 36 °C for three days under an ambience containing 5% (v/v) carbon dioxide gas. After this culture, the cells adhering to the culture vessel were separated with a sterilized silicone blade and recovered. The cells were centrifuged at 8,000 rpm for 30 minutes. The sediment obtained consequently was resuspended in SPG and the resultant suspension was put to storage at -70 °C.

(C) Purification of elementary body of *Chlamydia pneumoniae* YK41

The frozen suspension of HL cells infected with the *Chlamydia pneumoniae* YK41 preserved at -70 °C was melted and homogenized by the use of a homogenizer. The homogenate was centrifugally separated at 2,500 rpm for 10 minutes and the supernatant consequently formed was recovered. The sediment was again suspended in SPG and treated in the same manner as described above to recover a new supernatant. This procedure was repeated twice more. The successive supernatants were joined into one volume.

Separately, in a centrifuging tube, a 0.03M tris-hydrochloride buffer (pH 7.4) containing 50% (w/v) sucrose was placed, then a mixed solution of 3 parts by volume of urografin 76% (produced by Schering Corporation) with 7 parts by volume of 0.03M tris hydrochloride buffer (pH 7.4) was superposed, and subsequently the supernatant recovered as described above was attentively superposed on the layer of the mixed solution. The superposed layers in the centrifuging tube were centrifuged at 8,000 rpm for one hour. The layer of the 0.03M tris hydrochloride buffer (pH 7.4) containing 50% (w/v) sucrose and the sediment were recovered from the tube. The recovered solution and SPG added thereto in an equal volume were subjected to centrifugation at 10,000 rpm for 30 minutes. From the resultant separated phases, the supernatant was discarded and the sediment was suspended in SPG. In the centrifuging tubes, continuous density-gradient solutions consisting 35% to 50% of Urografin 76% (produced by Schering Corporation) in 0.03M tris hydrochloride buffer (pH 7.4) (ratios by volume of the former component to the total volume of solution) were placed and the suspension mentioned above was superposed thereon. The superposed layers in the tubes were centrifuged at 8,000 rpm for one hour. When a small amount of the yellowish white band was sampled and observed under an electron microscope, it was found to contain the elementary body of *Chlamydia pneumoniae*. So, this band was recovered and diluted with SPG to twice the original volume, and centrifuged at 10,000 rpm for 30 minutes. The sediment obtained in consequence of the centrifugation was suspended in SPG, assayed for protein concentration (with the aid of a protein analysis kit produced by Biorad Corp, with bovine serum albumin as a standard), and put to storage at -70 °C.

(D) Preparation of genome DNA of *Chlamydia pneumoniae* YK-41 strain

Three hundred (300) µl of a suspension of the elementary body of the purified *Chlamydia pneumoniae* YK-41 strain mentioned above (protein concentration: 1.37 mg/ml) was centrifuged at 4 °C at 12,000 rpm for five minutes. The resultant sediment was suspended in 500 µl of 10 mM tris buffer (pH 8.0) containing 1 mM EDTA (hereinafter referred to as "TE buffer"). The same centrifugation was repeated and the resultant sediment was suspended in 300 µl of TE buffer. The produced suspension and 30 µl of an aqueous 2% SDS solution and 30 µl of an aqueous solution of 1 mg/ml proteinase K added thereto were incubated at 56 °C for 30 minutes to effect solution of the elementary body. The incubated solution and 350 µl of phenol-saturated 0.1M tris hydrochloride buffer (pH 8.0) added thereto were thoroughly stirred with a vortex mixer. The resultant mixture was centrifuged at 4 °C at 12,000 rpm for five minutes. From the separated

layers, the aqueous layer was recovered (for extraction of DNA). This procedure of extraction was repeated once more. The aqueous layer and 2 µl of a 10 mg/ml RNase solution added thereto were incubated at 37 °C for two hours to effect decomposition of RNA. The incubated solution and 300 µl of a mixed solution consisting of a phenol-saturated 0.1M tris-hydrochloride buffer (pH 8.0), chloroform, and isoamyl alcohol at a volumetric ratio of 25 : 24 : 1 (hereinafter referred to as "PCI") were thoroughly stirred with a vortex mixer. The resultant mixture was centrifuged at 4 °C at 12,000 rpm for five minutes. From the separated layers, the aqueous layer was recovered. This procedure was repeated until a fifth time.

One part by volume of the resultant solution and 1/10 part by volume of an aqueous 10M ammonium acetate solution and two parts by volume of ethanol added thereto were left standing for five minutes to effect precipitation of DNA. The resultant mixed solution was centrifuged at 4 °C at 12,000 rpm for five minutes. The sediment plus 600 µl of an aqueous 70% ethanol solution was thoroughly stirred and centrifuged at 4 °C at 12,000 rpm for five minutes to effect purification. This procedure was repeated twice more. The contents of the centrifuging tubes were left standing for 15 minutes with the lids of the tubes kept open to dry the sediment. The dry sediment was dissolved with 200 µl of TE and the resultant solution was put to storage at -20 °C.

(E) Preparation of genome DNA expression library

One hundred (100) µl of a genome DNA solution and 10 µl of a restriction endonuclease grade M-buffer and 10 µl of a restriction endonuclease mixed solution (obtained by mixing 0.4 µl each of *AccI*, *Hae III*, and 1/50 dilution *AluI* with 20 µl of TE) added thereto were left reacting at 37 °C for 20 minutes. The reaction time of 20 minutes mentioned above was a duration necessary for DNA to be decomposed into partially digested DNA fractions of sizes ranging from 1 kbp through 7 kbp. It was empirically found in advance by using a small amount of genome DNA. The resultant reaction solution and 100 µl of PCI added thereto were thoroughly stirred with a vortex mixer and the produced mixture was centrifuged at 4 °C at 12,000 rpm for five minutes. The aqueous phase was recovered from the separated layers consequently obtained. The recovered aqueous layer and 10 µl of an aqueous 3M sodium acetate solution and 220 µl of ethanol added thereto were left standing at -80 °C for 15 minutes to effect precipitation of partially digested DNA. The produced mixed solution was centrifuged at 4 °C at 12,000 rpm for five minutes. From the separated layers, the supernatant was discarded. The sediment was mixed with 600 µl of an aqueous 70% ethanol solution and the produced mixture was again centrifuged at 12,000 rpm for five minutes. The supernatant was discarded and the sediment was dried under a reduced pressure.

The partially digested DNA consequently obtained was dissolved in 20 µl of purified water. The amount 19 µl of the DNA solution and 14 µl of a linker (20 pmole/µl) represented by the following base sequence, 4.5 µl of 10 mM ATP, 4.5 µl of a 0.2M tris-hydrochloride buffer (pH 7.6; hereinafter referred to as "tenfold concentration ligation grade buffer") containing 50 mM $MgCl_2$, 50 mM dithiothreitol, and 500 µg/ml bovine serum albumin, 2 µl of purified water, and 1 µl of T4 ligase added thereto were left reacting at 16 °C for four hours to effect addition of the linker.

5'-AATTCGAACCCCTTCG-3'

3'-GCTTGGGAAGCp-5'

The partially digested DNA adding the linker as described above was treated with a column (Chroma Spin 6000) using a 10 mM tris-hydrochloride buffer containing 0.1M NaCl and 1 mM EDTA as a migration phase. From the eluate, fractions each of two drops were separated. Each fraction was partly analyzed by 0.8% agarose gel electrophoresis to recover a fraction containing DNA segments of sizes from 1 kbp through 7 kbp. The amount 144 µl of the produced fraction and 13 µl of purified water, 20 µl of 10 mM ATP, 20 µl of a 0.5M tris-hydrochloride buffer (pH 7.6 maximum; hereinafter referred to as "tenfold concentration phosphorylation grade buffer") containing 0.1M $MgCl_2$, 50 mM dithiothreitol, 1 mM spermidine hydrochloride, and 1 mM EDTA, and 3 µl of T4 polynucleotide kinase added thereto were left reacting at 37 °C for 30 minutes to effect phosphorylation of the 5' terminal of the DNA fragment. The resultant reaction solution and 200 µl of PCI added thereto were thoroughly mixed by shaking. The produced mixture was centrifuged at 4 °C at 12,000 rpm for five minutes. From the separated layers, the aqueous layer was recovered. The aqueous phase was made to precipitate nucleotide by addition of 1 µl of an aqueous 20 mg/ml glycogen solution, 20 µl of an aqueous 3M sodium acetate solution, and 400 µl of ethanol. The produced solution was centrifuged at 4 °C at 12,000 rpm for 10 minutes. The supernatant was discarded. The sediment was mixed with 200 µl of 70% ethanol and again centrifuged. From the separated layers, the supernatant was discarded. The sediment was air dried and then dissolved in, 1 µl of purified water.

The amount 0.6 µl of the resultant aqueous solution and 1 µl of λ gIII DNA (1 µg/µl, produced by Stratagene Corp.) cleaved in advance with a restriction endonuclease *EcoRI*, 0.5 µl of a tenfold concentration ligation grade buffer, 0.5 µl of 10 mM ATP, 0.4 µl of T4 ligase, and 2 µl of purified water added thereto were left reacting overnight at 4 °C. Then, the recombinant λ gIII DNA consequently obtained was packaged by the use of a packaging kit (produced by Stratagene Corp. and marketed under trademark designation of Gigapack II Gold").

(F) Production of *Chlamydia pneumoniae*-specific monoclonal antibody

Cultivation and transfer of the myeloma cell strain

The myeloma cell strain used for the production of the monoclonal antibody was P3/NS1/1-Ag 4-1 (ATCC TIB-18). It was incubated and subjected to successive transfer culture in the RPMI 1640 culture medium containing 10% (v/v) bovine fetal serum. Two weeks prior to the cell fusion, the strain was incubated for one week in the RPMI 1640 culture medium containing 0.13 mM of 8-azaguanine, 0.5 µg/ml of a mycoplasma expellant (produced by Dainippon Pharmaceutical Co., Ltd. and marketed under product code of "MC-210"), and 10% (v/v) bovine fetal serum and then it was incubated in a standard culture medium for one week.

Immunization of mouse

Two hundred (200) µl of the suspension of the aforementioned elementary body having a protein concentration of 270 µg/ml was centrifuged at 12000 rpm for 10 minutes. The precipitate and 200 µl of PBS added thereto were together suspended. The suspension was emulsified by the addition of 100 µl of Freund's adjuvant. A portion, 150 µl in volume, of the emulsion was hypodermally injected into the back of a mouse (0th day of experiment). On the 14th, 34th, and 49th day, the suspension of the purified elementary body having a protein concentration of 270 µg/ml was intra-abdominally injected in a fixed dose of 100 µl into the mouse. Further, 50 µl of the suspension of the purified elementary body having a protein concentration of 800 µg/ml was intra-abdominally injected into the mouse on the 69th day and 100 µl of the same suspension was similarly injected into the mouse on the 92nd day. On the 95th day, the mouse was sacrificed to extract the spleen, which was put to use in the cell fusion.

Cell fusion

In a round bottom glass tube, 10⁸ spleen cells obtained from the spleen of the immunized mouse and 10⁷ myeloma cells were thoroughly mixed and centrifuged at 1400 rpm for five minutes. The supernatant was removed and the remaining cells were further mixed thoroughly. The cells and 0.4 ml of the RPMI 1640 culture medium containing 30% (w/v) polyethylene glycol and kept in advance at 37°C were together left standing at rest for 30 seconds. The resultant mixture was centrifuged at 700 rpm for six minutes. The glass tube containing this mixture and 10 ml of the RPMI 1640 culture medium added anew thereto was slowly rotated to ensure thorough dispersion of polyethylene glycol and centrifuged at 1400 rpm for five minutes. The supernatant was completely removed. The precipitate and 5 ml of the HAT culture medium added thereto were together left standing at rest for five minutes. The resultant mixture and 10 - 20 ml of the HAT culture medium added thereto were together left standing at rest for 30 minutes and then diluted by the addition of the HAT culture medium until the myeloma cell concentration reached 3.3 x 10⁵/ml to suspend the cells. The suspension was dispensed two drops each to the wells of a 96-well plastic incubation vessel by the use of a Pasteur's pipet. The suspension was incubated in the atmosphere of 5% (v/v) carbon dioxide gas at 36°C. After one day, 7 days, and 14 days following the start of the incubation, the HAT culture medium was added one to two drops each to the wells.

Screening of antibody-producing cells

The purified elementary body of the *Chlamydia pneumoniae* YK 41 strain was solubilized with 1% (w/v) SDS, dialyzed against a 0.05M sodium bicarbonate buffer solution (pH 9.6) containing 0.02% of sodium azide, diluted until the protein concentration reached a level in the range of 1 - 10 µg/ml, dispensed 50 µl each to the wells of a 96-well EIA grade plate made of vinyl chloride, and left standing at rest overnight at 4°C to induce adsorption of the antigen. The supernatant was removed. 150 µl of the PBS containing 0.02% (w/v) Tween 20 was added to the wells and the plate was left standing at rest for three minutes. The wells were deprived of the PBS and cleaned. After the wells were given a cleaning treatment once more, 100 µl of the PBS containing 1% (v/v) bovine serum albumin was added to the wells and left standing at rest overnight at 4°C to effect blocking. The wells were deprived of the PBS containing the bovine serum albumin, cleaned twice in the same manner as above with the PBS containing 0.02% (w/v) Tween 20 and, after adding 50 µl of the culture supernatant of the fused cells, left at rest at room temperature for two hours. The wells were cleaned three times in the same manner as above with the PBS containing 0.02% (w/v) Tween 20 and, after adding 50 µl of the goat anti-mouse IgG antibody (25 ng/ml) labeled with peroxidase, left standing at rest at room temperature for two hours. The wells were cleaned three times in the same manner as above with the PBS containing 0.02% (w/v) Tween 20 and, after adding 50 µl of the ABTS solution (produced by KPL Corp.), left standing at rest at room temperature for 15 minutes - one hour to induce a coloring reaction. The contents of the wells were tested for absorbance at 405 nm by the use of a 96-well EIA plate grade photometer.

As a result, positive wells were detected and the supernatants of culture broth in these wells were found to contain an antibody capable of reacting the elementary body. The cells in these wells were recovered severally with the Pas-

teur's pipet, transferred to a 24-well plastic incubation vessel and, after adding 1 - 2 ml of the HAT culture medium, incubated in the same manner as above.

Cloning by limiting dilution method

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The fused cells propagated in the 24-well plastic incubation vessel were tested for cell concentration and diluted with the HT culture medium to adjust the number of cells to 20/ml. Separately, the thymocytes of 4- to 6-week old mice suspended in the HT culture medium were dispensed to a 96-well plastic culture vessel at a rate of 2×10^5 /well and, after adding the aforementioned fused cells (cell concentration 20/ml) at a rate of 50 μ l/well, incubated in an atmosphere of 5% (v/v) carbon dioxide gas at 36°C. After 1 day, 7 days, and 14 days following the start of the incubation, the HT culture medium was added to the culture vessel at a rate of 1 to two drops/well. From the wells observed to have propagated cells, the supernatant of the culture broth was recovered in a fixed volume of 50 μ l per well and then analyzed in the same manner as above to confirm the production of an antibody.

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From the wells in which only one cell colony was present, cells producing an antibody able to react with the elementary body and showing quick propagation were recovered and allowed to continue propagation in a 24-well plastic culture vessel. The same cloning procedure was repeated until a hybridoma AY6E2E8 was ultimately obtained.

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Production of monoclonal antibody

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The hybridoma AY6E2E8 was cultured in a 75 cm² plastic cell culture flask holding therein 20 ml of the RPMI 1640 culture medium containing 10% (v/v) bovine fetal serum. From the culture broth formed in the flask, a sample, 16 - 18 ml in volume, was extracted at intervals of three to four days. The residual culture broth was meanwhile replenished to a total volume of 20 ml with a fresh supply of the RPMI 1640 culture medium containing 10% (v/v) bovine fetal serum. Thus, the subculture of the hybridoma was continued. The samples extracted from the culture broth were centrifuged at 1200 rpm for five minutes to recover the supernatant (the culture supernatant containing the monoclonal antibody).

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To a Balb/c mouse which had received intra-abdominal injection of 0.5 ml of pristane two weeks in advance of the experiment, the hybridoma strain suspended in the PBS at a concentration of $1 - 5 \times 10^6$ /ml was intra-abdominally injected in a volume of 1 ml. After three weeks thence, the ascites was recovered from the Balb/c mouse and centrifuged at 1200 rpm for five minutes to recover the supernatant (ascites containing the monoclonal antibody).

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Identification of subclass of monoclonal antibody

The subclass of the monoclonal antibody was identified with the ISOTYPE Ab-STAT (produced by Sang Stat Medical Corp.). As a result, the subclass of the monoclonal antibody produced by the hybridoma AY6E2E8 was identified to be IgG2b.

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Purification of monoclonal antibody

The monoclonal antibody produced by the hybridoma AY6E2E8 was purified as follows. A mixture of 1 part by volume of the monoclonal antibody-containing ascites obtained by injecting the hybridoma AY6E2E8 intra-abdominally to the mouse with 3 parts by volume of PBS was centrifuged at 3000 rpm for ten minutes. The resultant supernatant was passed through a filter, 0.22 μ m in pore size. The filtrate was purified by the HPLC using Chromatop Superprotein A Column (4.6 mm Diam. x 100 mm, produced by NGK Insulators Ltd. This column was equilibrated with the PBS in advance of the treatment.

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A sample, 1 ml in volume, of the filtrate emanating from the 0.22 μ m filter was injected into the column. The column was washed by passing the PBS first at a flow rate of 1 ml/min for three minutes and then at a flow rate of 5 ml/min for four minutes. The monoclonal antibody adsorbed on the column was eluted by passing a solution of 8.77 g of NaCl, 16.7 g of citric acid (monohydrate), and 14.72 g of Na₂HPO₄ · 12H₂O in 1 liter of purified water through the interior of the column at a flow rate of 2 ml/min for five minutes. The fractions of the desorbed monoclonal antibody were gathered and diluted with a TTBS solution.

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The elementary body of Chlamydia pneumoniae was dissolved to obtain the peptide contained in the elementary body. The peptide and the monoclonal antibody mentioned above were subjected to the Western blotting to determine the specificity of the acquired monoclonal antibody.

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As a result, the acquired monoclonal antibody was found to be capable of recognizing the Chlamydia pneumoniae 53 kDa antigen polypeptide.

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A hybridoma 70 was acquired in the same manner as the hybridoma AY6E2E8. When the monoclonal antibody producing the hybridoma 70 was tested for specificity by following the procedure described above, it was found that this monoclonal antibody was capable of recognizing the Chlamydia pneumoniae 73 kDa antigen polypeptide.

When the monoclonal antibody produced by the hybridoma 70 was examined in the same manner as above by way

of identification of subclass, the subclass of this antibody was found to be IgG.

(G) Cloning of DNA coding for antigenic polypeptide

5 One platinum loop full of the Y1090r-strain of *Escherichia coli* was inoculated to an LB (containing 5 g of NaCl, 10 g of polypeptone, and 5 g of yeast extract per liter of water) culture medium containing 0.2% maltose and 50 µg/ml of ampicillin and shaken cultured at 37 °C overnight. The resultant culture solution was centrifuged at 2,000 rpm for 10 minutes. The sediment (*Escherichia coli*) was mixed with 9 ml of an aqueous 10 mM MgSO₄ solution. The amount 0.35 ml of the *Escherichia coli* suspension and 0.1 to 10 µl of the λ gt11 (DNA library) suspension added thereto were incubated at 37°C for 20 minutes to infect the *Escherichia coli* with λ gt11. The λ gt11-infected *Escherichia coli* mentioned above was added to 2.5 ml of a liquid LB agar culture medium kept warmed in advance at 47 °C and the resultant mixture was scattered on an LB agar culture medium. After the upper-layer culture medium was solidified, the entire culture medium was cultured at 42 °C for three to four hours. At the time that a plaque was observed, a nitrocellulose filter (containing perforations 82 mm in diameter) immersed in advance in an aqueous 10 mM IPTG solution was mounted in the upper-layer agar culture medium. Then, the whole culture medium was cultured at 37 °C for 12 hours. With a syringe having the tip of the nozzle thereof smeared with black ink, the filter was pierced at three asymmetrical points selected as marks on the filter. Then, the filter now bearing the marks of the black ink was extracted from the agar culture medium and washed three times with a 20 mM tris-hydrochloride buffer (pH 7.5) containing 150 mM NaCl and 0.1% Tween 20 (hereinafter referred to as "TTBS buffer"). The residual agar culture medium was put to storage in a refrigerator.

20 The filter was immersed in a 0.1% bovine serum albumin-containing solution of a 20 mM tris-hydrochloride buffer (pH 7.5) containing 150 mM NaCl (hereinafter referred to as "TBS buffer") and shaken at 37 °C for one hour to effect a blocking reaction thereon. Then, the filter was washed twice with the TTBS buffer, immersed in the 10 µg/ml TTBS solution of a monoclonal antibody specific to *Chlamydia pneumoniae*, and shaken at 37 °C for one hour. The filter was washed three times with the TTBS buffer and then shaken in a peroxidase-labelled anti-mouse IgG antibody solution (TTBS buffer, 50 ng/ml) at 37 °C for one hour. The filter was washed three times with the TTBS buffer and three times with the TBS buffer, then immersed in a color ground substance solution (prepared by adding 60 µl of an aqueous 30% hydrogen peroxide solution and 20 ml of a methanolic 0.3% 4-chloro-1-naphthol solution to 100 ml of the TBS buffer), and left standing therein at room temperature for about 30 minutes. At the time that the filter was thoroughly colored, this filter was extracted from the solution, washed with purified water, and air-dried.

30 The plaques formed on the agar culture medium at the positions corresponding to the colored spots on the filter were searched out and identified. The relevant portions of the agar were pierced with a Pasteur pipet to recover the plaques. Each recovered plaque was placed in a 50 mM tris-hydrochloride buffer (pH 7.5) containing 0.1 M NaCl, 8 mM magnesium sulfate, and 0.01% gelatin (hereinafter referred to as "SM buffer") and one drop of chloroform, and left standing therein at 4 °C overnight to effect extraction of the λ phage from the plaque. The procedure just described was repeated until the plaque wholly reacted with the monoclonal antibody mentioned above to obtain a clone of the DNA coding for the antigen polypeptide.

35 As a result, the λ phage which expressed a *Chlamydia pneumoniae*-specific antigen polypeptide reactive with a *Chlamydia pneumoniae*-specific monoclonal antibody was obtained and designated as 53-3S λ phage.

40 (H) Culture of 53-3S λ phage and purification of DNA

Plaques were formed by following the procedure described in (F) above. One of the plaques was recovered, placed in 100 µl of the SM buffer, and left standing therein at 4 °C overnight to effect extraction of the λ phage. In the LB culture medium in which 250 µl of the Y1090r- strain of *Escherichia coli* was cultured overnight, 5 to 10 µl of the λ phage solution was placed and left standing therein at 37 °C for 20 minutes to effect infection of the *Escherichia coli* with the λ phage. The infected *Escherichia coli* was inoculated to 50 ml of the LB culture medium containing 10 mM magnesium sulfate and kept warm in advance at 37 °C and shaken cultured therein at 37 °C for five to seven hours until the bacteriolysis of the *Escherichia coli* by the λ phage occurred. The resultant culture solution, after adding 250 µl of chloroform, was centrifuged at 3,000 rpm for 10 minutes to effect removal of the residual cells of *Escherichia coli* and obtain a suspension of the λ phage. The λ phage DNA was purified by the use of a special device (produced by Promega Corp. and marketed under trademark designation of "Wizard λ Preps Kit").

55 (I) Amplification of DNA coding for *Chlamydia pneumoniae* antigenic polypeptide

A 600 µl grade microtube was charged with 61.5 µl of purified water, 10 µl of a tenfold concentration of reaction buffer (a tris-hydrochloride buffer, pH 8.3, containing 500 mM KCl, 15 mM MgCl₂, and 0.01% gelatin), 1 µl of 20 mM dNTP, 0.1 µl of 53-3S λ phage DNA solution, 1 µl of 20 nM λ gt11 forward primer (produced by Takara Shuzo Co., Ltd.), 1 µl of 20 nM λ gt11 reverse primer (produced by Takara Shuzo Co., Ltd.), and 0.5 µl of AmpliTaq DNA Polymerase, with

two or three drops of mineral oil placed to form a top layer. The contents of the microtube were subjected to 30 circles of incubation, each consisting of 30 seconds' standing at 94 °C, 30 seconds' standing at 55 °C, and two minutes' standing at 73 °C to effect amplification of the DNA. After the reaction, the reaction solution was subjected to 1.2% low-melting temperature agarose gel electrophoresis to excise the amplified DNA. This amplified DNA was purified by the use of "Wizard PCR Prep Kit" (produced by Promega Corp.).

(J) Analysis for DNA base sequence

The analysis of the DNA for base sequence was effected by subjecting a sample to a sequence reaction in accordance with the fluorescence-labelled terminator cycle sequence method using a Taq DNA polymerase with a PCR-amplified DNA as a template and analyzing the reaction product by a DNA sequencer (produced by Applied Biosystems Corp. and marketed under product code of "Model 373A"). The DNA base sequence consequently obtained was examined by the gene sequence analysis soft (produced by Hitachi Software Engineering Co., Ltd. and marketed under trademark designation of "DNASIS") to estimate agglutination, ligation, and amino acid translation region. Consequently, the sequence was identified as SEQ ID No: 9.

The results of the analysis of the sequence of SEQ ID No: 9 show that about 60% of the amino acid sequence of the 53KDa antigenic polypeptide from the N terminal thereof toward the C terminal was elucidated.

The DNA which codes for the *Chlamydia pneumoniae* antigen polypeptide is specific to *Chlamydia pneumoniae* and it has been cloned by utilizing a monoclonal antibody recognizing the 53 Kda antigen polypeptide. Thus, this DNA apparently encodes the 53 kDa antigen polypeptide.

The search for homology of both the base sequence and the amino acid sequence of SEQ ID No: 9 was carried out in accordance with the GenBank data base confirmed absence of a known series exhibiting high homology.

Example 2: Preparation of recombinant vector containing DNA coding for polypeptide containing part of antigenic polypeptide of *Chlamydia pneumoniae*, and preparation of transformant carrying the vector.

Though the acquired DNA evidently coded for the 53 KDa antigen polypeptide as mentioned above, it was expressed as shown below to determine whether or not it would react with the antibody mentioned above by way of precaution.

A plasmid pBBK10MM was severed with restriction enzymes of BamHI and XhoI and subjected to 1.2% low melting temperature solution agarose gel electrophoresis to excise about 4.6 Kbp of DNA fragment. This fragment was purified. The synthetic DNA's of SEQ ID No: 11 and SEQ ID No: 12 were added each in an amount of 1 ng to 100 ng of the DNA fragment and they were ligated by the use of a DNA ligation kit (produced by Takara Shuzo Co., Ltd.) The resultant reaction product was placed in an *Escherichia coli* HB101 strain-competent cell (produced by Takara Shuzo Co., Ltd.) to prepare a transformant and acquire a plasmid, which was designated as pADA431. This plasmid was severed with a restriction enzyme MunI and then subjected to an alkali phosphatase reaction to effect removal of the 5' phosphoric acid base.

Separately, the 53-3S λ phage DNA was severed with a restriction enzyme EcoRI. One hundred (100) ng of the pADA431 plasmid DNA severed with the restriction enzyme MunI mentioned above was added to 50 ng of the DNA fragment and they were ligated in the same manner as described above to prepare a transformant and acquire a plasmid incorporating therein the restriction enzyme EcoRI fragment of 53-3S λ phage DNA, which was designated as pCPN533 α . This plasmid was a DNA of a length of about 5.7 kbp possessing a base sequence of SEQ ID No: 10 and was capable of expressing the polypeptide containing part of 53K antigenic polypeptide with a host *Escherichia coli*. The base sequence of the DNA coding for the polypeptide containing part of the 53K antigenic polypeptide was shown by SEQ ID No: 4. The amino acid sequence deduced from this base sequence was shown by SEQ ID No: 2. An *Escherichia coli* carrying the plasmid pCPN533a was subjected to culture, electrophoresis, transfer to a nitrocellulose membrane, and detection with a monoclonal antibody in the same manner as described above. As a result, the occurrence of a colored band corresponding to the polypeptide mentioned above was visually conformed. This fact indicates that the *Escherichia coli* carrying the plasmid pCPN533a expressed the 53K antigenic polypeptide capable of reacting with a monoclonal antibody specifically reactive with *Chlamydia pneumoniae*.

Example 3: Acquisition of DNA coding for the entire 53KDa antigenic polypeptide of *Chlamydia pneumoniae*

A DNA possessing base sequences of SEQ ID Nos. 26 and 27 was synthesized based on the base sequence of SEQ ID No. 9 by the use of a DNA synthesizing device.

Ten (10) μ l of the aqueous solution of genome DNA of the *Chlamydia pneumoniae* YK 41 strain (DNA content: about 1 μ g) obtained in Example 1 and 5 μ l of a K buffer concentrated to 1/10 times the original volume, 35 μ l of purified water, and 5 μ l of a limiting enzyme Hind III (19 U/ μ l) added thereto were kept together at 37°C for three hours.

The resultant reaction solution was extracted from phenol. The extract and ethanol added thereto were together

centrifuged to obtain a precipitate. This precipitate and 5 µl of the Hind III cassette DNA (20 ng/µl) in the PCR in vitro Cloning Kit (proprietary designation of Takara Shuzo Co., Ltd.) and 15 µl of ligation solution added thereto were kept together at 16°C for 30 minutes.

The resultant reaction solution was extracted from phenol. The extract and ethanol added thereto are centrifuged together to acquire a precipitate. This precipitate was dissolved in 10 µl of purified water.

The resultant solution and 78.5 µl of purified water, 10 µl of a PCR grade buffer concentrated to 1/10 times the original volume, 8 µl of 2.5 mM dNTP, and 0.5 µl (5 U/µl) of Taq polymerase added thereto and 1 µl of a DNA possessing the base sequence of SEQ ID No. 26 (20 pmol/µl) and 1 µl of a DNA possessing the base sequence of SEQ ID No. 28 (20 pmol/µl) (enclosed as Primer C1 in the aforementioned kit) further added thereto as primer DNA's were placed together in a microtube, 0.6 ml in volume, with two drops of mineral oil superposed on the resultant mixture in the microtube. The mixture was subjected to 30 temperature cycles each consisting of 30 seconds at 94°C, 2 minutes at 55°C, and 3 minutes at 72°C. This procedure will be referred to hereinafter as "PCR process."

One (1) µl of the reaction solution resulting from the PCR process and 1 µl of a DNA possessing the base sequence of SEQ ID No. 27 (20 pmol/µl) and 1 µl of a DNA possessing the base sequence of SEQ ID No. 29 (20 pmol/µl) (enclosed as Primer C2 in the aforementioned kit) added thereto as primer DNA's were subjected to the PCR process.

The reaction solution resulting from the second PCR process was subjected to electrophoresis with 1.2% low melting agarose gel to separate an agarose gel containing a DNA, about 1.4 kbp in size. The Wizard PCR Prep kit (Promega Corp) was used for the purification of the DNA. The separated agarose gel and the buffer solution enclosed in the kit were together heated to dissolve the agarose gel. The purifying resin enclosed in the kit was added to the resultant solution to adsorb the DNA. The resultant mixture was centrifuged to obtain the purifying resin as a precipitate. The precipitate was washed with propanol and centrifuged again to obtain a precipitate. Purifying water was added to the precipitate to dissolve the DNA out of the purifying resin. The resultant mixture was centrifuged to obtain a supernatant (aqueous DNA solution). The process described above will be referred to herein below as "DNA purifying process."

The acquired aqueous DNA solution was caused to undergo a sequence reaction by the fluorescence-labeled terminator sequence method using the Taq DNA polymerase templated by the contained DNA and was analyzed for the base sequence of DNA with a DNA sequencer, Model 373A, (Applied Biosystems Corp.). The DNA base sequence consequently obtained was compiled and ligated by the software for gene sequence analysis (produced by Hitachi Software Engineering Co., Ltd. and marketed under trademark designation of "DNASIS") to estimate the amino acid translation region. The process just described will be referred to herein below as "base sequence analyzing process."

When the acquired DNA was analyzed for base sequence, it was found that this DNA possessed about 50 bp of base sequences on the 3' terminal side of the DNA encoding the antigen polypeptide of *Chlamydia pneumoniae* acquired in Example 1. It was further found that about 0.7 kb of coding region containing a stop codon existed on the downstream side of the base sequence.

A DNA possessing the base sequence of SEQ ID No. 30 was synthesized as a primer corresponding to the upstream part of the DNA encoding the antigen polypeptide of *Chlamydia pneumoniae* based on the base sequence of SEQ ID No. 9 and a DNA possessing the base sequence of SEQ ID No. 31 was synthesized as a primer corresponding to the downstream part of the DNA encoding the antigen polypeptide of *Chlamydia pneumoniae* based on the base sequence containing the aforementioned about 0.7 kb of code zone severally by the use of the DNA synthesizer.

The PCR process was performed on 1 µl of the DNA possessing the base sequence of SEQ ID No. 30 DNA and 1 µl of the DNA possessing the base sequence of SEQ ID No. 31 as a primer DNA by using 1 µl of the aqueous solution of the genome DNA of the *Chlamydia pneumoniae* YK 41 strain obtained in Example 1.

The DNA purifying process mentioned above was carried out on the reaction solution resulting from the third round of the PCR process to obtain about 1.5 kbp of DNA.

The base sequence analyzing process mentioned above was carried out on the acquired aqueous solution of DNA. When the base sequence of the acquired DNA was analyzed, it was found that this DNA possessed the base sequence of SEQ ID No. 3 and encoded the amino acid sequence of SEQ ID No. 1.

DNA coding for the entire 53KDa antigenic polypeptide of *Chlamydia pneumoniae* was obtained by effecting a genome walking by the use of the plasmid pCPN533a and the DNA library of λ gtlI.

Example 4: Preparation of recombinant vector containing DNA coding for entire 53KDa antigenic polypeptide of *Chlamydia pneumoniae* and preparation of transformant carrying the vector

The recombination vector containing the DNA coding for the whole *Chlamydia pneumoniae* 53 kDa antigen polypeptide and the transformant containing the vector can be manufactured as follows.

A recombinant vector containing a DNA coding for the entire 53KDa antigenic polypeptide of *Chlamydia pneumoniae* and a transformant carrying the vector are prepared by following the procedure of Example 2 using the DNA coding for the entire 53KDa antigenic polypeptide of *Chlamydia pneumoniae*.

Example 5: Preparation of DNA coding for 73K antigenic polypeptide of Chlamydia pneumoniae

A hybridoma 70 was acquired by the same method as used for the acquisition of a hybridoma AY6E2E8. The murine ascites was acquired by using the hybridoma 70. The supernatant of the ascites was analyzed for the quality of the monoclonal antibody contained therein. The results of this analysis indicate that this monoclonal antibody was specific to the antigen polypeptide of 73 KDa of Chlamydia pneumoniae.

A clone 70-2S λ phage was obtained by following the procedure of Example 1 while using a monoclonal antibody 70 in the place of the monoclonal antibody SCP53 or AY6E2E8. From the phage, a sequence of SEQ ID No: 13 was obtained.

The results of the analysis of the sequence of SEQ ID No: 13 clearly indicate that about 90% of the amino acid sequence of the 73K antigenic protein of Chlamydia pneumoniae from the N terminal toward the C terminal thereof was clarified.

The search for homology of both the base sequence and the amino acid sequence of SEQ ID No: 13 was effected in accordance with the GenBank data base. The results of the search clearly show that these sequences exhibited high homology with the gene base sequence isolated from Chlamydia trachomatis [L. M. Sardinia et al: J. Bacteriol., Vol. 17., 335-341 (1989)].

Example 6: Production of anti-Chlamydia pneumoniae antibody using antigenic polypeptide of Chlamydia pneumoniae as antigen

The anti-Chlamydia pneumoniae antibody can be produced by using the antigen polypeptide of Chlamydia pneumoniae as follows.

(A) Culture and passage of myeloma cell strain

As a myeloma cell strain, P3X63Ag8.653 (ATCC CRL-1580) is cultured and passed in a RPMI1640 culture medium containing 10% (v/v) bovine fetal serum. Two weeks before the strain is subjected to cellular fusion, this strain is cultured for one week in the RPMI1640 culture medium containing 0.13 mM of 8-azaguanine, 0.5 μ g/ml of a mycoplasma removing agent (produced by Dainippon Pharmaceutical Co., Ltd. and marketed under product code of "MC-210"), and 10% (v/v) bovine fetal serum. The subsequent one week is spent for culture in an ordinary culture medium.

(B) Immunization of mouse

The amount 200 μ l of a solution of the antigenic polypeptide mentioned above and having a protein concentration of 270 μ g/ml is emulsified by addition of 200 μ l of a Freund's complete adjuvant. The produced emulsion is hypodermically injected in an amount of 150 μ l into the back of a mouse (the date of this injection reckoned as 0th day). On the 14th day, 34th day, and 49th day, 100 μ l of a suspension of the antigenic polypeptide having a protein concentration of 270 μ g/ml is intraabdominally injected into the mouse. Further, 50 μ l of a suspension of the same antigenic polypeptide having a protein concentration of 800 μ g/ml is intraabdominally injected into the mouse on the 69th day and 100 μ l of the same suspension injected intraabdominally to the mouse on the 92nd day. On the 95th day, the mouse is sacrificed to extract the spleen. This spleen is utilized for cellular fusion.

(C) Cellular fusion

In a round-bottom glass tube, 10^8 splenic cells obtained from the spleen mentioned above and 10^7 myeloma cells are thoroughly mixed. The resultant mixture is centrifuged at 1,400 rpm for five minutes and, with the consequently formed supernatant removed therefrom, further mixed thoroughly. The produced mixture is added to 0.4 ml of a RPMI1640 culture medium containing 30% (w/v) polyethylene glycol and kept warmed in advance at 37 °C and left standing therein for 30 seconds. The culture medium now containing the mixture is centrifuged at 700 rpm for six minutes. The glass tube, after adding 10 ml of the RPMI1640 culture medium, is gently rotated so as to permit thorough mixture of the polyethylene glycol. The mixture is then centrifuged at 1,400 rpm for five minutes. The supernatant consequently formed is thoroughly removed. The sediment and 6 ml of the HAT culture medium added thereto are left standing for five minutes. The resultant mixture and 10 to 20 ml of the HAT culture medium added thereto are left standing for 30 minutes. The HAT culture medium is further added thereto in such an amount as to set a myeloma cell concentration at 3.3×10^5 /ml to obtain a suspension of cells. The suspension is dispensed at a rate of two drops to each of the 96-well plastic culture vessel by the use of a Pasteur pipet. The suspension is cultured under an ambience of 5% (v/v) carbon dioxide gas at 36 °C. Then, one or two drops of the HAT culture medium are added to each of the wells after the elapse of one day, seven days, and 14 days.

(D) Screening of antibody-producing cells

The antigenic polypeptide mentioned above is suspended in a 0.05M sodium bicarbonate suspension (pH 9.6) containing 0.02% (w/v) sodium azide so as to set the protein concentration in the range of from 1 to 10 µg/ml. The resultant suspension is dialyzed against a 0.05M sodium bicarbonate buffer (pH 9.6) containing 0.02% of sodium azide. The dialyzed suspension is diluted so as to set the protein concentration in the range of from 1 to 10 µg/ml. The diluted dialyzed suspension is dispensed at a rate of 50 µl to each of the wells of a 96-well plate for EIA made of vinylchloride and left standing therein at 4 °C overnight to effect adsorption of the antigen. The supernatant consequently formed is removed from the wells. To each of the wells, 150 µl of PBS containing 0.02% (w/v) Tween 20 is added, left standing therein for three minutes, then removed, and washed. The washing is repeated once more. To the well, 100 µl of PBS containing 1% (v/v) bovine serum albumin is added and left standing at 4 °C overnight to effect blocking. The PBS containing the bovine serum albumin is removed and then washed twice more with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. Then, 50 µl of the culture supernatant of fused cells is added to the well and left standing therein at room temperature for two hours. The well is washed three times with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. In the well, 50 µl of a goat anti-mouse IgG antibody labelled with peroxidase (25 ng/ml) is placed and left standing at room temperature. The well is washed three times with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. In the well, 50 µl of an ABTS solution (produced by KPL Corp.) is placed and left standing at room temperature for 15 minutes to one hour to effect a reaction of coloration. The culture solution in the well is tested for absorbance at 405 nm with the photometer for 96-well EIA plate. The cells in the positive wells are severally recovered with the Pasteur pipet, transferred into a 24-well plastic culture vessel and, after adding 1 to 2 ml of the HAT culture medium, cultured in the same manner as described above.

(E) Cloning by limiting dilution method

The fused cells of two strains propagated in a 24-well plastic culture vessel are tested for cell concentration and severally diluted with a HT culture medium until the number of cells decreased to 20/ml. Separately, the thymocytes of four- to six-weeks old mice suspended in the HT culture medium are dispensed at a rate of 1 to 2 x 10⁵/well to a 96-well plastic culture vessel and the fused cells mentioned above (cell concentration 20/ml) are dispensed at a rate of 50 µl/well to the same culture vessel and cultured under an ambience of 5% (v/v) carbon dioxide gas at 36 °C. One day, seven days, and 14 days thereafter, the HT culture medium is added thereto at a rate of one to two drops per well. From each of the wells in which the growth of cells is observed, the culture supernatant is recovered in a fixed amount of 50 µl. This supernatant is analyzed in the same manner as in (D) titled "Screening of antibody-producing cells" to confirm the production of an antibody therein.

The cells which allowed the occurrence of a single cellular colony in a well, produced an antibody capable of reacting with an elementary body, and achieved quick proliferation are recovered from the relevant wells and are subsequently proliferated in a 24-well plastic culture vessel. Further, a hybridoma producing an anti-Chlamydia pneumoniae antibody is obtained by repeating the same cloning process as described above. This hybridoma is cultured and the anti-Chlamydia pneumoniae antibody is produced from the resultant culture supernatant.

Example 7: Detection and determination of anti-Chlamydia pneumoniae antibody using an antigenic polypeptide as an antigen

The anti-Chlamydia pneumoniae antibody can be detected and measured by using the antigen polypeptide of this invention as an antigen as follows.

The polypeptide formed of the amino acid sequence of SEQ ID No: 1 is used as an antigenic polypeptide. It is fixed on a microtiter plate, made to add a PBS containing bovine serum albumin, and left standing overnight at 4 °C to effect blocking. The PBS containing the bovine serum albumin was removed and the well is washed twice with the PBS containing 0.02% (w/v) Tween 20. The blood serum from a patient is added to the well thereto and is left standing at room temperature for two hours. The resultant solution is removed and the well is washed three times with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. In each of the wells, a peroxidase-labelled mouse anti-human IgG antibody is placed and left standing at room temperature for two hours. The solution in the well is removed and the well is washed three times with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. In the well, an ABTS solution (produced by KPL Corp.) is placed and left standing at room temperature for 15 minutes to one hour to effect a reaction of coloration. The solution is then tested for absorbance at 405 nm by the use of a photometer for 96-well EIA plate.

Example 8: Production of recombinant vector carrying DNA coding for fused protein of peptide containing DHFR and part of antigenic polypeptide of Chlamydia pneumoniae and production of transformant containing the recombinant vector

5 A plasmid pBBK10MM was severed with restriction enzymes of BamHI and XhoI and subjected to 1.2% low melting temperature solution agarose gel electrophoresis to excise about 4.6 Kbp of DNA fragment. This fragment was purified.

Separately, a 53-3S λ phage DNA was severed with a restriction enzyme EcoRI to obtain about 1.0 Kbp of DNA fragment similarly in a purified form. This DNA segment was further severed with a restriction enzyme Avall to obtain about 0.8 Kbp of a DNA segment similarly in a purified form. The amount 100 ng of about 4.6 Kbp of DNA segment, 10 100 ng of about 0.8 Kbp of DNA segment mentioned above, and 1 ng of each of the synthetic DNA's of SEQ ID Nos: 21 through 24 added thereto were subjected to DNA ligation by the use of the DNA ligation kit (produced by Takara Shuzo Co., Ltd.). The reaction product was placed in an Escherichia coli HB101 strain competent cell (produced by Takara Shuzo Co., Ltd.) to produce a transformant.

This transformant was spread on a LB agar culture medium containing 50 mg/L of ampicillin and cultured thereon 15 at 37 °C for 24 hours. The Escherichia coli colony consequently obtained was inoculated to 3 ml of the LB culture medium containing 50 mg/L of ampicillin and then shaken cultured overnight at 37 °C. The plasmid vector was separated from the culture medium by the alkali lysis method, severed with a restriction enzyme NruI, and analyzed by 0.8% agarose gel electrophoresis to select an Escherichia coli possessing a recombinant plasmid vector which had produced DNA segments of 616 bp and 4822 bp. The recombinant plasmid vector thus obtained was designated as pCPN533T.

20 This plasmid vector was a DNA of a length of about 5.4 kbp possessing a base sequence of SEQ ID No: 25. It was capable of expressing a fused protein having a polypeptide containing part of the 53KDa antigenic polypeptide of Chlamydia pneumoniae ligated to the C terminal of DHFR. The base sequence of the DNA coding for this fused protein was shown by SEQ ID No: 18. The amino acid sequence deduced from this base sequence was shown by SEQ ID No: 16.

25 Example 9: Recognition of fused protein of polypeptide containing DHFR and part of 53KDa antigenic polypeptide of Chlamydia pneumoniae

One platinum loop full of the HB101 strain of Escherichia coli retaining plasmid pCPN533T was inoculated to 3 ml 30 of the LB culture medium containing 50 mg/l of ampicillin and shaken cultured overnight at 37°C. The amount 10 μ l of the culture medium containing the Escherichia coli and 10 μ l of loading buffer (a 0.156M tris-hydrochloride buffer containing 0.01% of bromophenol blue, 10% of mercapto ethanol, 20% of glycerol, and 5% of SDS and having pH 6.8) added thereto were heated at 80 °C for five minutes. The resultant reaction solution was subjected to 5-20% polyacrylamide gradient gel electrophoresis. On the anode plate of a semi-dry blotting device, one filter paper wetted with a 0.3M tris aqueous solution containing 10% of methanol and 0.05% sodium dodecyl sulfate, one filter paper wetted with a 25 mM tris aqueous solution containing 10% of methanol and 0.05% of sodium dodecyl sulfate, one filter paper wetted with a 25 mM tris aqueous solution containing 10% of methanol and 0.05% of sodium dodecyl sulfate, one nitrocellulose membrane wetted with a 25 mM tris aqueous solution containing 10% of methanol, 0.05% of sodium dodecyl sulfate, and 40 mM aminocaproic acid, the polyacryl amide gel completely undergone the aforementioned electrophoresis and 40 two filter papers wetted with a 25 mM tris aqueous solution containing 40 mM aminocaproic acid were superposed sequentially in the order mentioned. A cathode plate was set as opposed to the anode plate across the superposed filters and an electric current was passed through the filters at a current density of 2.5 mA/cm² for one hour to effect transfer of the protein in the polyacrylamide gel to the nitrocellulose membrane. The nitrocellulose membrane was placed in a TBS buffer containing 0.1% of bovine serum albumin and left standing therein at room temperature for not less than 45 one hour to effect blocking. The nitrocellulose membrane was washed twice with the TTBS buffer and then shaken in a monoclonal antibody solution produced by the hybridoma SCP53 (in the 5 to 10 μ g/ml TTBS buffer) at 37 °C for one hour. The nitrocellulose membrane was washed three times with the TTBS buffer and then shaken in an aqueous solution of an anti-mouse IgG antibody labelled with peroxidase (in the 50 ng/ml TTBS buffer) at 37 °C for one hour. The nitrocellulose membrane was washed three times with the TTBS buffer and then placed in a coloring ground substance 50 solution (obtained by mixing 100 ml of the TBS buffer with 60 μ l of an aqueous 30% hydrogen peroxide solution, and 20 ml of a methanolic solution of 4-chloro-1-naphthol) and left reacting at room temperature for 30 minutes. The nitrocellulose membrane was extracted, washed with purified water, and then air-dried. As a result, colored bands were observed at positions corresponding to sizes of fused protein. This fact indicates that the Escherichia coli possessing the plasmid pCPN533T expressed the fusion protein containing 53KDa antigen capable of reacting with the monoclonal 55 antibody specifically reacting Chlamydia pneumoniae.

Example 10: Acquisition of DNA coding for entire 53KDa antigenic polypeptide of Chlamydia pneumoniae

The DNA encoding the whole 53 kDa antigen polypeptide of Chlamydia pneumoniae was already acquired in

Example 3. However, it was separately obtained the DNA as follows.

A DNA coding for the entire 53KDa antigenic polypeptide of Chlamydia pneumoniae was also obtained by effecting a genome walking by the use of the plasmid pCPN533T and the DNA library of λ gt11. When these DNAs were analyzed for base sequence, it was found to possess the 484th through 1947th base sequences of SEQ ID No: 17 and code for the 162nd through 649th amino sequences of SEQ ID No: 15.

Example 11: Production of recombinant vector carrying DNA coding for fused protein of DHFR and entire 53KDa antigenic polypeptide of Chlamydia pneumoniae and production of transformant containing the recombinant vector

The recombinant vector containing the DNA encoding the fused protein of DHFR and the whole 53 kDa antigen polypeptide of Chlamydia pneumoniae and the transformant containing the recombinant vector can be produced as follows.

A recombinant vector containing a DNA coding for the fused protein of the DHFR and the entire 53KDa antigenic polypeptide of Chlamydia pneumoniae is produced by following the procedure of Example 8 while using a DNA coding for the plasmid pBBK10MM and the entire 53KDa antigenic polypeptide of Chlamydia pneumoniae mentioned above and the transformant containing the recombinant vector was produced. The base sequence of the DNA coding for the fused protein is shown by SEQ ID No: 17 and the amino acid sequence deduced from this base sequence is shown by SEQ ID No: 15.

Example 12: Production of anti-Chlamydia pneumoniae antibody by use of fused protein as an antigen

The anti-Chlamydia pneumoniae antibody can be produced by using the fused protein of this invention as an antigen as follows.

A hybridoma producing an anti-Chlamydia pneumoniae antibody is obtained by following the procedure of Example 6 while using the fused protein mentioned above as an antigen for immunization. This hybridoma is cultured and the anti-Chlamydia pneumoniae antibody is produced from the culture supernatant consequently formed.

Example 13: Detection and determination of anti-Chlamydia pneumoniae antibody by using fused protein as antigen

The anti-Chlamydia pneumoniae can be detected and measured by using the fused protein of this invention as an antigen as follows.

The polypeptide formed of the amino acid sequence of SEQ ID No: 15 is used as a fused protein. It is fixed on a microtiter plate, made to add a PBS containing bovine serum albumin, and left standing overnight at 4 °C to effect blocking. The PBS containing the bovine serum albumin is removed and the plate is washed twice with the PBS containing 0.02% (w/v) Tween 20. The blood serum from a patient is added to the wells and is left standing at room temperature for two hours. The well is washed three times with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. In each of the wells, a peroxidase-labelled mouse anti-human IgG antibody is placed and left standing at room temperature for two hours. The culture solution in the well is washed three times with the PBS containing 0.02% (w/v) Tween 20 in the same manner as described above. In the well, an ABTS solution (produced by KPL Corp.) is placed and left standing at room temperature for 15 minutes to one hour to effect a reaction of coloration. The culture solution is then tested for absorbance at 405 nm by the use of a photometer for 96-well EIA plate.

Example 14: Detection of Chlamydia pneumoniae gene by PCR method

A DNA formed of a base sequence of SEQ ID No: 19 and a DNA formed of a base sequence of SEQ ID No: 20 were chemically synthesized with a DNA synthesizing device produced by Applied Biosystems Corp and were designated respectively as Primer 53F2 and Primer 53R2.

The cells infected with the YK41 strain of Chlamydia pneumoniae or the L2 strain of Chlamydia trachomatis or the Bugd. 17-SL strain of Chlamydia psittaci were recovered by centrifugation. The cells plus 0.1 ml of a 50 mM tris-hydrochloride buffer (pH 8.3) containing 50 mM of KCl, 2.5 mM of $MgCl_2$, 0.1 mg/ml of gelatin, 0.45% of Nonidet P40, 0.45% of Tween 20, and 0.1 mg/ml of proteinase K were kept warmed at 56 °C for one hour and then heated at 95 °C for 10 minutes to inactivate the proteinase K and obtain a sample containing the gene of relevant chlamydia.

One (1) μ l of the sample was combined with 78.5 μ l of purified water, 8 μ l of an aqueous 2.5 mM dNTP solution, 10 μ l of a 100 mM tris-hydrochloride buffer (pH 8.3) containing 500 mM of KCl and 15 mM of $MgCl_2$, 1 μ l each of the aqueous solutions of 30 μ M Primer 53F2 and Primer 53R2 mentioned above, and 0.5 μ l of 5 U/ μ l of Taq polymerase. The resultant mixture was superposed by 50 μ l of mineral oil and subjected to 30 cycles of a procedure which consisted of heating at 94 °C for 30 seconds, at 60 °C for 30 seconds, and at 72 °C for 60 seconds, cooling, and warming.

After the reaction was completed, 2 μ l of the reaction solution was subjected to agarose gel electrophoresis, with the gel immersed in 0.5 μ g/ml of ethidium bromide to make a band of DNA visible by irradiation of an ultraviolet light.

As a result, the sample obtained from the YK41 strain of Chlamydia pneumoniae was found to form a visible band of DNA of a size of 360 bp corresponding to a region interposed between the base sequence of Primer 53F2 and a base sequence complementary to the base sequence of Primer 53R2 in all the base sequences of SEQ ID No: 3. The samples obtained from the other strains were not found to form any visible band of DNA.

INDUSTRIAL APPLICABILITY

The antigenic polypeptide of this invention formed of a polypeptide A containing at least five continuous amino acid sequences in the polypeptides of SEQ ID No: 1 can be utilized as for the examination of an antibody of Chlamydia pneumoniae.

The antigenic polypeptide of this invention the polypeptide A of which is a polypeptide arising from the loss of 1 to 250 amino acids from the polypeptides of SEQ ID No: 1 has an amino acid sequence of a small length and, therefore, is enabled to increase the number of antigenic peptides which can be fixed as on a carrier. Thus, it can be utilized for the production of a diagnostic agent of high sensitivity.

The antigenic polypeptide of this invention the polypeptide A of which is a polypeptide resulting from the substitution of 1 to 100 amino acids in the polypeptides of SEQ ID No: 1 by other amino acids is capable of forming a structure only sparingly susceptible of the decomposition by a protease and, therefore, is excellent in stability as an antigen.

The antigenic polypeptide of this invention the polypeptide A of which is a polypeptide having an amino acid or 2 to 1000 amino acid sequences ligated to at least five continuous amino acid sequences in the polypeptides of SEQ ID No: 1 can be fixed as to a carrier by making use of the amino acid or 2 to 1000 amino acid sequences and, therefore, does not easily yield to decline or loss of the antigenicity by fixation.

The antigenic polypeptide of this invention the polypeptide A of which is a polypeptide formed of amino acid sequences of SEQ ID No: 1 possesses the whole of antigenic polypeptides specific to Chlamydia pneumoniae and, therefore, is highly suitable for the examination of antigens and for accurate diagnosis of infections involving Chlamydia pneumoniae.

The antigenic polypeptide of this invention the polypeptide A of which is a polypeptide formed of amino acid sequences of SEQ ID No: 2 or ID No: 5 possesses an antigenic part specific to Chlamydia pneumoniae and, therefore, is highly suitable for the examination of antigens and for accurate diagnosis of infections involving Chlamydia pneumoniae.

The DNA of this invention which is a DNA coding for any of the antigenic polypeptides mentioned above or a DNA complementary thereto can be utilized for the production of an antigenic polypeptide suitable for the examination of antigens of Chlamydia pneumoniae, the diagnosis of infections involving Chlamydia pneumoniae, and the like.

The DNA of this invention the base sequence of which is a base sequence of SEQ ID No: 3 codes for the whole of the antigenic polypeptide specific to Chlamydia pneumoniae can be utilized for the production of an antigenic polypeptide suitable for the examination of antibodies specific to Chlamydia pneumoniae.

The DNA of this invention the base sequence of which is a base sequence of SEQ ID No: 4 or ID No: 7 codes for the antigenic part specific to Chlamydia pneumoniae can be utilized for the production of an antigenic polypeptide suitable for the examination of antigens specific to Chlamydia pneumoniae.

The recombinant vector of this invention containing any of the DNA's mentioned above can be utilized for the production of an antigenic polypeptide suitable for the examination of an antibody of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

The recombinant vector of this invention which is a pCPN533a plasmid possessing a base sequence of SEQ ID No: 10 is capable of expressing a polypeptide possessing an antigenic part specific to Chlamydia pneumoniae and, therefore, can be utilized for the production of an antigenic polypeptide highly suitable as for the examination of antibodies specific to Chlamydia pneumoniae.

The transformant of this invention which contains any of the recombinant vectors mentioned above can be utilized for the production of an antigenic polypeptide suitable as for the examination of antibody specific to Chlamydia pneumoniae.

The method of this invention for the production of an anti-Chlamydia pneumoniae antibody which is characterized by using any of the antigenic polypeptides mentioned above as an antigen can be utilized for the production of a diagnostic agent for infections involving Chlamydia pneumoniae.

The method of this invention for the detection and determination of an anti-Chlamydia pneumoniae antibody which is characterized by using any of the antigenic polypeptides mentioned above as an antigen can be utilized for the examination of antibodies of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

Particularly when an antigenic polypeptide having an amino acid sequence of a small length is utilized, it manifests high sensitivity because it allows an increase in the number of antigenic polypeptides to be fixed as on a carrier.

When an antigenic polypeptide having amino acids inherent therein substituted by other amino acids is utilized for the detection and determination mentioned above, the results of the detection and determination are highly reliable because the antigenic polypeptide is capable of forming a structure only sparingly susceptible to decomposition by a

protease and, consequently, excellent in stability.

When an antigenic polypeptide adding other amino acid sequences is utilized for the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the role ideally because it enables a polypeptide being used as an antigen to be fixed as on a carrier by making use of amino acids or 2 to 1000 amino acid sequences and only sparingly incurs decline or loss of the antigenicity due to the fixation.

When an antigenic polypeptide formed of amino acid sequences of SEQ ID No: 1 is utilized for the examination of antibodies or the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the examination or the diagnosis with perfect accuracy because a polypeptide being used as an antigen possesses the whole antigenic polypeptide specific to Chlamydia pneumoniae.

When an antigenic polypeptide formed of amino acid sequences of SEQ ID No: 2 or ID No: 5 is utilized for the examination of antibodies or the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the examination or the diagnosis with perfect accuracy because a polypeptide being used as an antigen possesses an antigenic part specific to Chlamydia pneumoniae.

The reagent of this invention for the detection and determination of an anti-Chlamydia pneumoniae antibody which contains any of the antigenic polypeptides mentioned above as an antigen ideally fits the examination of antibodies of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

Particularly, when an antigenic polypeptide having an amino acid sequence of a small length is utilized for the reagent, the reagent enjoys high sensitivity because it allows an increase in the number of antigenic polypeptides to be fixed as on a carrier.

When an antigenic polypeptide having amino acids inherent therein substituted by other amino acids is utilized for the detection and determination mentioned above, the results of the examination and determination are highly reliable because the antigenic polypeptide is capable of forming a structure only sparingly susceptible to decomposition by a protease and, as a result, excellent in stability.

Further, when an antigenic polypeptide adding other amino acid sequences is utilized for the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the role ideally because it enables a polypeptide being used as an antigen to be fixed as on a carrier by making use of amino acids or 2 to 1000 amino acid sequences and only sparingly incurs decline or loss of the antigenicity due to the fixation.

Then, when an antigenic polypeptide formed of amino acid sequences of SEQ ID No: 1 is utilized for the examination of antibodies or the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the examination or the diagnosis with perfect accuracy because a polypeptide being used as an antigen possesses the whole antigenic polypeptide specific to Chlamydia pneumoniae.

When an antigenic polypeptide formed of amino acid sequences of SEQ ID No: 2 or ID No: 5 is utilized for the examination of antibodies or the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the examination or the diagnosis with perfect accuracy because a polypeptide being used as an antigen possesses an antigenic part specific to Chlamydia pneumoniae.

The diagnostic agent of this invention which has any of the antigenic polypeptides mentioned above as an active component ideally fits the diagnosis of infections involving Chlamydia pneumoniae.

Particularly, when an antigenic polypeptide having an amino acid sequence of a short length is adopted for the agent, the agent enjoys high sensitivity because it allows an increase in the number of antigenic polypeptides to be fixed as on a carrier.

When an antigenic polypeptide having amino acids inherent therein substituted by other amino acids is utilized for the detection and determination mentioned above, the results of the examination and determination are highly reliable because the antigenic polypeptide is capable of forming a structure only sparingly susceptible to decomposition by a protease and, as a result, excellent in stability.

Further, when an antigenic polypeptide adding other amino acid sequences is utilized for the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the role ideally because it enables a polypeptide being used as an antigen to be fixed as on a carrier by making use of amino acids or 2 to 1000 amino acid sequences and only sparingly incurs decline or loss of the antigenicity due to the fixation.

Then, when an antigenic polypeptide formed of amino acid sequences of SEQ ID No: 1 is utilized for the examination of antibodies or the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the examination or the diagnosis with perfect accuracy because a polypeptide being used as an antigen possesses the whole antigenic polypeptide specific to Chlamydia pneumoniae.

When an antigenic polypeptide formed of amino acid sequences of SEQ ID No: 2 or ID No: 5 is utilized for the examination of antibodies or the diagnosis of infections involving Chlamydia pneumoniae, it fulfills the examination or the diagnosis with perfect accuracy because a polypeptide being used as an antigen possesses an antigenic part specific to Chlamydia pneumoniae.

The fused protein of this invention which has ligated to a polypeptide of SEQ ID No: 14 either directly or through the medium of an amino acid sequence a polypeptide A containing at least five continuous amino acid sequences in the polypeptides of SEQ ID No: 1 can be utilized as for the examination of antibodies of Chlamydia pneumoniae.

The fused protein of this invention the polypeptide A of which is a polypeptide arising from the loss of 1 to 250 amino acids from the polypeptides of SEQ ID No: 1 has an amino acid sequence of a small length and, therefore, is enabled to increase the number of antigenic peptides which can be fixed as on a carrier. Thus, it can be utilized for the production of a diagnostic agent of high sensitivity.

5 The fused protein of this invention the polypeptide A of which is a polypeptide resulting from the substitution of 1 to 100 amino acids in the polypeptides of SEQ ID No: 1 by other amino acids is capable of forming a structure only sparingly susceptible of the decomposition by a protease and, therefore, is excellent in stability as an antigen.

The fused protein of this invention which is a polypeptide formed of amino acid sequences of SEQ ID No: 15 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because it possesses the whole of antigenic polypeptides specific to Chlamydia pneumoniae.

10 The fused protein of this invention which is a polypeptide formed of amino acid sequences of SEQ ID No: 16 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because it possesses an antigenic part specific to Chlamydia pneumoniae.

The DNA of this invention which is a DNA coding for any of the fused proteins mentioned above or a DNA complementary thereto can be utilized for the production of a fused protein suitable for the examination of antibodies of Chlamydia pneumoniae, the diagnosis of infections involving Chlamydia pneumoniae, and the like.

15 The DNA of this invention the base sequences of which are base sequences of SEQ ID No: 17 can be utilized for the production of a fused protein suitable as for the examination of antibodies specific to Chlamydia pneumoniae because the fused protein coded for by this DNA possesses the whole of antigenic polypeptides specific to Chlamydia pneumoniae.

20 The DNA of this invention the base sequences of which are base sequences of SEQ ID No: 18 can be utilized for the production of a fused protein suitable as for the examination of antibodies specific to Chlamydia pneumoniae because the fused protein coded for by this DNA possesses an antigenic part specific to Chlamydia pneumoniae.

25 The recombinant vector of this invention which carries any of the DNA's mentioned above can be utilized for the production of a fused protein suitable for the examination of antibodies of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

The recombinant vector of this invention which is a pCPN533T plasmid can be utilized for the production of a fused protein highly suitable as for the examination of antibodies specific to Chlamydia pneumoniae because it is capable of expressing a fused protein possessing an antigenic part specific to Chlamydia pneumoniae.

30 The transformant of this invention which contains any of the recombinant vectors mentioned above can be utilized for the production of a fused protein suitable as for the examination of antibodies specific to Chlamydia pneumoniae.

The method of this invention for the production of an anti-Chlamydia pneumoniae antibody which is characterized by using any of the fused proteins mentioned above as an antigen can be utilized for the production of a diagnostic agent for infections involving Chlamydia pneumoniae.

35 The method of this invention for the detection and determination of an anti-Chlamydia pneumoniae antibody which is characterized by using any of the fused proteins mentioned above as an antigen is suitable for the examination of antibodies of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

40 Particularly, when a fused protein having an amino acid sequence of a short length is adopted for the method, the method enjoys high sensitivity because this fused protein allows an increase in the number of antigenic polypeptides to be fixed as on a carrier.

When a fused protein having amino acids inherent therein substituted by other amino acids is utilized for the detection and determination mentioned above, the results of the examination and determination are highly reliable because the fused protein is capable of forming a structure only sparingly susceptible to decomposition by a protease and, as a result, excellent in stability.

45 A fused protein which is formed of amino acid sequences of SEQ ID No: 15 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because a fused protein being used as an antigen possesses the whole of antigenic polypeptides specific to Chlamydia pneumoniae.

A fused protein which is formed of amino acid sequences of SEQ ID No: 16 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because a fused protein being used as an antigen possesses an antigenic part specific to Chlamydia pneumoniae.

50 The reagent of this invention which contains any of the fused proteins mentioned above as an antigen is suitable for the examination of antibodies of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

55 Particularly, when a fused protein having an amino acid sequence of a small length is utilized for the reagent, the reagent enjoys high sensitivity because it allows an increase in the number of antigenic polypeptides to be fixed as on a carrier.

When a fused protein having amino acids inherent therein substituted by other amino acids is utilized for the detection and determination mentioned above, the results of the examination and determination are highly reliable because the fused protein is capable of forming a structure only sparingly susceptible to decomposition by a protease and, as a

result, excellent in stability.

A fused protein which is formed of amino acid sequences of SEQ ID No: 15 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because a fused protein being used as an antigen possesses the whole of antigenic polypeptides specific to Chlamydia pneumoniae.

A fused protein which is formed of amino acid sequences of SEQ ID No: 16 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because a fused protein being used as an antigen possesses an antigenic part specific to Chlamydia pneumoniae.

The diagnostic medicine of this invention having any of the fused proteins mentioned above as an active component thereof is suitable for the examination of antibodies of Chlamydia pneumoniae and the diagnosis of infections involving Chlamydia pneumoniae.

Particularly, when a fused protein having an amino acid sequence of a small length is utilized for the agent, the agent enjoys high sensitivity because it allows an increase in the number of antigenic polypeptides to be fixed as on a carrier.

When a fused protein having amino acids inherent therein substituted by other amino acids is utilized for the detection and determination mentioned above, the results of the examination and determination are highly reliable because the fused protein is capable of forming a structure only sparingly susceptible to decomposition by a protease and, as a result, excellent in stability.

A fused protein which is formed of amino acid sequences of SEQ ID No: 15 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because a fused protein being used as an antigen possesses the whole of antigenic polypeptides specific to Chlamydia pneumoniae.

A fused protein which is formed of amino acid sequences of SEQ ID No: 16 is highly suitable for the examination of antibodies and the diagnosis of infections involving Chlamydia pneumoniae because a fused protein being used as an antigen possesses an antigenic part specific to Chlamydia pneumoniae.

The probe and the primer of this invention are suitable for the detection and determination of a Chlamydia pneumoniae gene and the diagnosis of infections involving Chlamydia pneumoniae.

Particularly, a probe and a primer which possesses base sequences of SEQ ID No: 19 or ID No: 20 can be utilized for accurate diagnosis of infections involving Chlamydia pneumoniae because they possess base sequences specific to Chlamydia pneumoniae.

The method of this invention for the detection and determination of a Chlamydia pneumoniae gene by the use of any of the probes or primers mentioned above is suitable for the diagnosis of infections involving Chlamydia pneumoniae.

The reagent of this invention for the detection and determination of a Chlamydia pneumoniae which contains any of the probes or the primers mentioned above is ideally suitable for the diagnosis of infections involving Chlamydia pneumoniae.

The diagnostic agent of this invention which has any of the probes or the primers mentioned above as an active component is ideally suitable for the diagnosis of infections involving Chlamydia pneumoniae.

Sequence Listing

5 INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:488 amino acids

10 (B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile Met
1 5 10 15

Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp Lys
20 25 30

Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly Lys
25 35 40 45

Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly Lys
30 50 55 60

Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln Gly
65 70 75 80

Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala Asp
35 85 90 95

Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala Thr
40 100 105 110

Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met Glu
115 120 125

Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys Glu
45 130 135 140

Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly Ser
50 145 150 155 160

Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro Arg

55

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	165	170	175
	Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln Thr		
5	180	185	190
	Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr Gln		
	195	200	205
10	Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala Ile		
	210	215	220
	Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala Glu		
15	225	230	235
	Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr Val		
	245	250	255
20	Met Ile Ala Val Ser Val Ala Ile Thr Val Ile Ser Ile Val Ala Ala		
	260	265	270
25	Ile Phe Thr Cys Gly Ala Gly Leu Ala Gly Leu Ala Ala Gly Ala Ala		
	275	280	285
	Val Gly Ala Ala Ala Ala Gly Gly Ala Ala Gly Ala Ala Ala Thr		
30	290	295	300
	Thr Val Ala Thr Gln Ile Thr Val Gln Ala Val Val Gln Ala Val Lys		
	305	310	315
35	Gln Ala Val Ile Thr Ala Val Arg Gln Ala Ile Thr Ala Ala Ile Lys		
	325	330	335
	Ala Ala Val Lys Ser Gly Ile Lys Ala Phe Ile Lys Thr Leu Val Lys		
40	340	345	350
	Ala Ile Ala Lys Ala Ile Ser Lys Gly Ile Ser Lys Val Phe Ala Lys		
45	355	360	365
	Gly Thr Gln Met Ile Ala Lys Asn Phe Pro Lys Leu Ser Lys Val Ile		
	370	375	380
50	Ser Ser Leu Thr Ser Lys Trp Val Thr Val Gly Val Gly Val Val Val		
	385	390	395
			400
55			

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Ala Ala Pro Ala Leu Gly Lys Gly Ile Met Gln Met Gln Leu Ser Glu
405 410 415
5 Met Gln Gln Asn Val Ala Gln Phe Gln Lys Glu Val Gly Lys Leu Gln
420 425 430
10 Ala Ala Ala Asp Met Ile Ser Met Phe Thr Gln Phe Trp Gln Gln Ala
435 440 445
Ser Lys Ile Ala Ser Lys Gln Thr Gly Glu Ser Asn Glu Met Thr Gln
15 450 455 460
Lys Ala Thr Lys Leu Gly Ala Gln Ile Leu Lys Ala Tyr Ala Ala Ile
465 470 475 480
20 Ser Gly Ala Ile Ala Gly Ala Ala
485 488

25

INFORMATION FOR SEQ ID NO: 2:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 271 amino acids

(B) TYPE: amino acid

35 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile Met
40 1 5 10 15
Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp Lys
20 25 30
45 Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly Lys
35 40 45
50 Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly Lys
50 55 60
Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln Gly

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	65	70	75	80
	Val	Ala	Ala	Gly
	Lys	Glu	Ser	Ser
	Glu	Ser	Gln	Lys
	Ala	Gly	Ala	Asp
5		85	90	95
	Thr	Gly	Val	Ser
	Gly	Ala	Ala	Ala
	Thr	Thr	Ala	Ser
	Asn	Thr	Ala	Thr
	100	105	110	
10	Lys	Ile	Ala	Met
	Gln	Thr	Ser	Ile
	Glu	Glu	Ala	Ser
	Lys	Ser	Met	Glu
	115	120	125	
15	Ser	Thr	Leu	Glu
	Ser	Leu	Gln	Ser
	Leu	Ser	Ala	Ala
	Gln	Met	Lys	Glu
	130	135	140	
	Val	Glu	Ala	Val
	Val	Val	Ala	Ala
	Leu	Ser	Gly	Lys
	Ser	Ser	Gly	Ser
20	145	150	155	160
	Ala	Lys	Leu	Glu
	Thr	Pro	Glu	Leu
	Pro	Lys	Pro	Gly
	Val	Thr	Pro	Arg
	165	170	175	
25	Ser	Glu	Val	Ile
	Glu	Ile	Gly	Leu
	Ala	Leu	Ala	Lys
	Ala	Ile	Gln	Thr
	180	185	190	
	Leu	Gly	Glu	Ala
	Thr	Lys	Ser	Ala
	Leu	Ser	Asn	Tyr
	Ala	Ser	Thr	Gln
30	195	200	205	
	Ala	Gln	Ala	Asp
	Gln	Thr	Asn	Lys
	Leu	Gly	Leu	Glu
	Lys	Gln	Ala	Ile
	210	215	220	
35	Lys	Ile	Asp	Lys
	Glu	Arg	Glu	Glu
	Tyr	Gln	Glu	Met
	Lys	Ala	Ala	Glu
	225	230	235	240
40	Gln	Lys	Ser	Lys
	Asp	Leu	Glu	Gly
	Thr	Met	Asp	Thr
	Val	Asn	Thr	Val
	245	250	255	
	Met	Ile	Ala	Lys
	Gly	Phe	Glu	Leu
	Pro	Trp	Gly	Pro
	Leu	Ile	Asn	
45	260	265	270	271

50 INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

55

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(A) LENGTH:1464 base pairs

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: double

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

10 ATG TCT ATT TCA TCT TCT TCA GGA CCT GAC AAT CAA AAA AAT ATC ATG 48

15 Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile Met

1 5 10 15

TCT CAA GTT CTG ACA TCG ACA CCC CAG GGC GTG CCC CAA CAA GAT AAG 96

20 Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp Lys

20 25 30

25 CTG TCT GGC AAC GAA ACG AAG CAA ATA CAG CAA ACA CGT CAG GGT AAA 144

30 Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly Lys

35 40 45

AAC ACT GAG ATG GAA AGC GAT GCC ACT ATT GCT GGT GCT TCT GGA AAA 192

35 Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly Lys

50 55 60

40 GAC AAA ACT TCC TCG ACT ACA AAA ACA GAA ACA GCT CCA CAA CAG GGA 240

45 Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln Gly

65 70 75 80

GTT GCT GCT GGG AAA GAA TCC TCA GAA AGT CAA AAG GCA GGT GCT GAT 288

50 Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala Asp

85 90 95

55

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ACT GGA GTA TCA GGA GCG GCT GCT ACT ACA GCA TCA AAT ACT GCA ACA 336

5 Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala Thr
100 105 110

AAA ATT GCT ATG CAG ACC TCT ATT GAA GAG GCG AGC AAA AGT ATG GAG 384

10 Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met Glu
115 120 125

15 TCT ACC TTA GAG TCA CTT CAA AGC CTC AGT GCC GCG CAA ATG AAA GAA 432

20 Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys Glu
130 135 140

GTC GAA GCG GTT GTT GTT GCT GCC CTC TCA GGG AAA AGT TCG GGT TCC 480

25 Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly Ser
145 150 155 160

30 GCA AAA TTG GAA ACA CCT GAG CTC CCC AAG CCC GGG GTG ACA CCA AGA 528

35 Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro Arg
165 170 175

TCA GAG GTT ATC GAA ATC GGA CTC GCG CTT GCT AAA GCA ATT CAG ACA 576

40 Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln Thr
180 185 190

45 TTG GGA GAA GCC ACA AAA TCT GCC TTA TCT AAC TAT GCA AGT ACA CAA 624

50 Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr Gln
195 200 205

GCA CAA GCA GAC CAA ACA AAT AAA CTA GGT CTA GAA AAG CAA GCG ATA 672

55

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Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala Ile
210 215 220
5 AAA ATC GAT AAA GAA CGA GAA GAA TAC CAA GAG ATG AAG GCT GCC GAA 720

Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala Glu
10 225 230 235 240
CAG AAG TCT AAA GAT CTC GAA GGA ACA ATG GAT ACT GTC AAT ACT GTG 768

Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr Val
15 245 250 255
20 ATG ATC GCG GTT TCT GTT GCC ATT ACA GTT ATT TCT ATT GTT GCT GCT 816

Met Ile Ala Val Ser Val Ala Ile Thr Val Ile Ser Ile Val Ala Ala
25 260 265 270
ATT TTT ACA TGC GGA GCT GGA CTC GCT GGA CTC GCT GCG GGA GCT GCT 864

Ile Phe Thr Cys Gly Ala Gly Leu Ala Gly Leu Ala Ala Gly Ala Ala
30 275 280 285
35 GTA GGT GCA GCG GCA GCT GGA GGT GCA GCA GGA GCT GCT GCC GCA ACC 912

Val Gly Ala Ala Ala Ala Gly Gly Ala Ala Gly Ala Ala Ala Ala Thr
40 290 295 300
ACG GTA GCA ACA CAA ATT ACA GTT CAA GCT GTT GTC CAA GCG GTG AAA 960

Thr Val Ala Thr Gln Ile Thr Val Gln Ala Val Val Gln Ala Val Lys
45 305 310 315 320
50 CAA GCT GTT ATC ACA GCT GTC AGA CAA GCG ATC ACC GCG GCT ATA AAA 1008

55

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Gln Ala Val Ile Thr Ala Val Arg Gln Ala Ile Thr Ala Ala Ile Lys
325 330 335
5 GCG GCT GTC AAA TCT GGA ATA AAA GCA TTT ATC AAA ACT TTA GTC AAA 1056

Ala Ala Val Lys Ser Gly Ile Lys Ala Phe Ile Lys Thr Leu Val Lys
10 340 345 350
GCG ATT GCC AAA GCC ATT TCT AAA GGA ATC TCT AAG GTT TTC GCT AAG 1104

Ala Ile Ala Lys Ala Ile Ser Lys Gly Ile Ser Lys Val Phe Ala Lys
15 355 360 365
20 GGA ACT CAA ATG ATT GCG AAG AAC TTC CCC AAG CTC TCG AAA GTC ATC 1152

Gly Thr Gln Met Ile Ala Lys Asn Phe Pro Lys Leu Ser Lys Val Ile
25 370 375 380
TCG TCT CTT ACC AGT AAA TGG GTC ACG GTT GGG GTT GGG GTT GTA GTT 1200

Ser Ser Leu Thr Ser Lys Trp Val Thr Val Gly Val Gly Val Val Val
30 385 390 395 400
35 GCG GCG CCT GCT CTC GGT AAA GGG ATT ATG CAA ATG CAG CTC TCG GAG 1248

Ala Ala Pro Ala Leu Gly Lys Gly Ile Met Gln Met Gln Leu Ser Glu
40 405 410 415
ATG CAA CAA AAC GTC GCT CAA TTT CAG AAA GAA GTC GGA AAA CTG CAG 1296

Met Gln Gln Asn Val Ala Gln Phe Gln Lys Glu Val Gly Lys Leu Gln
45 420 425 430
50 GCT GCG GCT GAT ATG ATT TCT ATG TTC ACT CAA TTT TGG CAA CAG GCA 1344

Ala Ala Ala Asp Met Ile Ser Met Phe Thr Gln Phe Trp Gln Gln Ala
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435 440 445

AGT AAA ATT GCC TCA AAA CAA ACA GGC GAG TCT AAT GAA ATG ACT CAA 1392

5

Ser Lys Ile Ala Ser Lys Gln Thr Gly Glu Ser Asn Glu Met Thr Gln

450 455 460

10 AAA GCT ACC AAG CTG GGC GCT CAA ATC CTT AAA GCG TAT GCC GCA ATC 1440

Lys Ala Thr Lys Leu Gly Ala Gln Ile Leu Lys Ala Tyr Ala Ala Ile

465 470 475 480

15 AGC GGA GCC ATC GCT GGC GCA GCA 1464

20

Ser Gly Ala Ile Ala Gly Ala Ala

485 488

25

INFORMATION FOR SEQ ID NO: 4:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:813

35 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATG TCT ATT TCA TCT TCT TCA GGA CCT GAC AAT CAA AAA AAT ATC ATG 48

45 Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile Met

1 5 10 15

50 TCT CAA GTT CTG ACA TCG ACA CCC CAG GGC GTG CCC CAA CAA GAT AAG 96

Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp Lys

55

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	20	25	30	
5	CTG TCT GGC AAC GAA ACG AAG CAA ATA CAG CAA ACA CGT CAG GGT AAA			144
	Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly Lys			
10	35 40 45			
	AAC ACT GAG ATG GAA AGC GAT GCC ACT ATT GCT GGT GCT TCT GGA AAA			192
15	Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly Lys			
	50 55 60			
	GAC AAA ACT TCC TCG ACT ACA AAA ACA GAA ACA GCT CCA CAA CAG GGA			240
20	Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln Gly			
	65 70 75 80			
25	GTT GCT GCT GGG AAA GAA TCC TCA GAA AGT CAA AAG GCA GGT GCT GAT			288
30	Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala Asp			
	85 90 95			
	ACT GGA GTA TCA GGA GCG GCT GCT ACT ACA GCA TCA AAT ACT GCA ACA			336
35	Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala Thr			
	100 105 110			
40	AAA ATT GCT ATG CAG ACC TCT ATT GAA GAG GCG AGC AAA AGT ATG GAG			384
45	Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met Glu			
	115 120 125			
	TCT ACC TTA GAG TCA CTT CAA AGC CTC AGT GCC GCG CAA ATG AAA GAA			432
50	Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys Glu			
	130 135 140			
55				

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	GTC GAA GCG GTT GTT GTT GCT GCC CTC TCA GGG AAA AGT TCG GGT TCC	480
5	Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly Ser	
	145 150 155 160	
10	GCA AAA TTG GAA ACA CCT GAG CTC CCC AAG CCC GGG GTG ACA CCA AGA	528
	Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro Arg	
15	165 170 175	
	TCA GAG GTT ATC GAA ATC GGA CTC GCG CTT GCT AAA GCA ATT CAG ACA	576
20	Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln Thr	
	180 185 190	
25	TTG GGA GAA GCC ACA AAA TCT GCC TTA TCT AAC TAT GCA AGT ACA CAA	624
	Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr Gln	
30	195 200 205	
	GCA CAA GCA GAC CAA ACA AAT AAA CTA GGT CTA GAA AAG CAA GCG ATA	672
35	Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala Ile	
	210 215 220	
40	AAA ATC GAT AAA GAA CGA GAA GAA TAC CAA GAG ATG AAG GCT GCC GAA	720
	Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala Glu	
45	225 230 235 240	
	CAG AAG TCT AAA GAT CTC GAA GGA ACA ATG GAT ACT GTC AAT ACT GTG	768
50	Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr Val	
	245 250 255	
55	ATG ATC GCG AAG GGG TTC GAA TTG CCA TGG GGG CCC TTA ATT AAT	813

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Met Ile Ala Lys Gly Phe Glu Leu Pro Trp Gly Pro Leu Ile Asn
 260 265 270 271

INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 259 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile Met

1 5 10 15

Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp Lys

20 25 30

Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly Lys

35 40 45

Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly Lys

50 55 60

Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln Gly

65 70 75 80

Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala Asp

85 90 95

Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala Thr

100 105 110

Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met Glu

115 120 125

Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys Glu

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130 135 140
Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly Ser
5 145 150 155 160
Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro Arg
10 165 170 175
Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln Thr
180 185 190
15 Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr Gln
195 200 205
Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala Ile
20 210 215 220
Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala Glu
225 230 235 240
25 Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr Val
245 250 255
Met Ile Ala
30 259

35

INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

40

(A) LENGTH: 571 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

50

Met Pro Lys Gln Ala Glu Tyr Thr Trp Gly Ser Lys Lys Ile Leu Asp

1 5 10 15

Asn Ile Glu Cys Leu Thr Glu Asp Val Ala Glu Phe Lys Asp Leu Leu

55

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	20	25	30
	Tyr Thr Ala His Arg Ile Thr Ser Ser Glu Glu Glu Ser Asp Asn Glu		
5	35	40	45
	Ile Gln Pro Gly Ala Ile Leu Lys Gly Thr Val Val Asp Ile Asn Lys		
	50	55	60
10	Asp Phe Val Val Val Asp Val Gly Leu Lys Ser Glu Gly Val Ile Pro		
	65	70	75
	Met Ser Glu Phe Ile Asp Ser Ser Glu Gly Leu Val Leu Gly Ala Glu		
15	85	90	95
	Val Glu Val Tyr Leu Asp Gln Ala Glu Asp Glu Glu Gly Lys Val Val		
	100	105	110
20	Leu Ser Arg Glu Lys Ala Thr Arg Gln Arg Gln Trp Glu Tyr Ile Leu		
	115	120	125
25	Ala His Cys Glu Glu Gly Ser Ile Val Lys Gly Gln Ile Thr Arg Lys		
	130	135	140
	Val Lys Gly Gly Leu Ile Val Asp Ile Gly Met Glu Ala Phe Leu Pro		
30	145	150	155
	Gly Ser Gln Ile Asp Asn Lys Lys Ile Lys Asn Leu Asp Asp Tyr Val		
	165	170	175
35	Gly Lys Val Cys Glu Phe Lys Ile Leu Lys Ile Asn Val Glu Arg Arg		
	180	185	190
40	Asn Ile Val Val Ser Arg Arg Glu Leu Leu Glu Ala Glu Arg Ile Ser		
	195	200	205
	Lys Lys Ala Glu Leu Ile Glu Gln Ile Ser Ile Gly Glu Tyr Arg Lys		
45	210	215	220
	Gly Val Val Lys Asn Ile Thr Asp Phe Gly Val Phe Leu Asp Leu Asp		
	225	230	235
50	Gly Ile Asp Gly Leu Leu His Ile Thr Asp Met Thr Trp Lys Arg Ile		
	245	250	255
55			

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Arg His Pro Ser Glu Met Val Glu Leu Asn Gln Glu Leu Glu Val Ile
260 265 270
5 Ile Leu Ser Val Asp Lys Glu Lys Gly Arg Val Ala Leu Gly Leu Lys
275 280 285
10 Gln Lys Glu His Asn Pro Trp Glu Asp Ile Glu Lys Lys Tyr Pro Pro
290 295 300
Gly Lys Arg Val Leu Gly Lys Ile Val Lys Leu Leu Pro Tyr Gly Ala
15 305 310 315 320
Phe Ile Glu Ile Glu Glu Gly Ile Glu Gly Leu Ile His Ile Ser Glu
325 330 335
20 Met Ser Trp Val Lys Asn Ile Val Asp Pro Ser Glu Val Val Asn Lys
340 345 350
Gly Asp Glu Val Glu Ala Ile Val Leu Ser Ile Gln Lys Asp Glu Gly
25 355 360 365
Lys Ile Ser Leu Gly Leu Lys Gln Thr Glu Arg Asn Pro Trp Asp Asn
30 370 375 380
Ile Glu Glu Lys Tyr Pro Ile Gly Leu His Val Asn Ala Glu Ile Lys
380 385 390 395
35 Asn Leu Thr Asn Tyr Gly Ala Phe Val Glu Leu Glu Pro Gly Ile Glu
400 405 410
Gly Leu Ile His Ile Ser Asp Met Ser Trp Ile Lys Lys Val Ser His
40 415 420 425
Pro Ser Glu Leu Phe Lys Lys Gly Asn Ser Val Glu Ala Val Ile Leu
430 435 440
45 Ser Val Asp Lys Glu Ser Lys Lys Ile Thr Leu Gly Val Lys Gln Leu
445 450 455
Ser Ser Asn Pro Trp Asn Glu Ile Glu Ala Met Phe Pro Ala Gly Thr
50 460 465 470 475
Val Ile Ser Gly Val Val Thr Lys Ile Thr Ala Phe Gly Ala Phe Val
55

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GTTGCTGCTG GGAAAGAATC CTCAGAAAGT CAAAAGGCAG GTGCTGATAC TGGAGTATCA 300
 5 GGAGCGGCTG CTACTACAGC ATCAAATACT GCAACAAAAA TTGCTATGCA GACCTCTATT 360
 10 GAAGAGGCGA GCAAAAGTAT GGAGTCTACC TTAGAGTCAC TTCAAAGCCT CAGTGCCGCG 420
 CAAATGAAAG AAGTCGAAGC GGTGTGTTGT GCTGCCCTCT CAGGGAAAAG TTCGGGTTCC 480
 15 GCAAAATTGG AAACACCTGA GCTCCCCAAG CCCGGGGTGA CACCAAGATC AGAGGTTATC 540
 20 GAAATCGGAC TCGCGCTTGC TAAAGCAATT CAGACATTGG GAGAAGCCAC AAAATCTGCC 600
 TTATCTAACT ATGCAAGTAC ACAAGCACAA GCAGACCAA CAAATAAACT AGGTCTAGAA 660
 25 AAGCAAGCGA TAAAAATCGA TAAAGAACGA GAAGAATACC AAGAGATGAA GGCTGCCGAA 720
 30 CAGAAGTCTA AAGATCTCGA AGGAACAATG GATACTGTCA ATACTGTGAT GATCGCG 777

35

INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

40

(A) LENGTH:1712 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

45

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

50

ATGCCAAAAC AAGCTGAATA TACTTGGGGA TCTAAAAAAA TTCTGGACAA TATAGAATGC 60

55

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	CTCACAGAAG ACGTTGCCGA ATTTAAAGAT TTGCTTTATA CGGCACACAG AATTACTTCG	120
5	AGCGAAGAAG AATCTGATAA CGAAATACAG CCTGGCGCCA TCCTAAAAGG TACCGTAGTT	180
10	GATATTAATA AAGACTTTGT CGTAGTTGAT GTTGGTCTGA AGTCTGAGGG AGTGATCCCT	240
	ATGTCAGAGT TCATAGACTC TTCAGAAGGT TTAGTGCTTG GAGCTGAAGT AGAAGTCTAT	300
15	CTCGACCAAG CCGAAGACGA AGAGGGCAAA GTTGTCTTT CTAGAGAAA AGCCACACGA	360
20	CAACGTCAAT GGAATACAT CTTAGCTCAT TGTGAAGAAG GTTCTATTGT TAAAGGTCAA	420
25	ATTACACGTA AAGTCAAAG CGGCCTTATT GTAGATATTG GAATGGAAGC CTTCTACCT	480
	GGATCACAAA TTGACAACAA GAAATCAAA AATTTAGATG ATTATGTCGG AAAAGTTGT	540
30	GAATTCAAAA TTTTAAAAAT TAACGTTGAA CGTCGCAATA TTGTTGTCTC AAGAAGAGAA	600
35	CTCTTAGAAG CTGAGAGAAT CTCTAAGAAA GCCGAACCTA TTGAACAAAT TTCTATCGGA	660
	GAATACCGCA AAGGAGTTGT TAAAAACATT ACTGACTTTG GTGTATTCTT AGATCTCGAT	720
40	GGTATTGACG GTCTTCTCCA CATTACCGAT ATGACCTGGA AGCGCATACG ACATCCTTCC	780
45	GAAATGGTCG AATTGAATCA AGAGTTGGAA GTAATTATTT TAAGCGTAGA TAAAGAAAAA	840
50	GGACGAGTTG CTCTAGGTCT CAAACAAAAA GAGCATAATC CTTGGGAAGA TATTGAGAAG	900
55	AAATACCCTC CTGGAACACG AGTTCTTGGT AAAATTGTGA AGCTTCTCCC CTACGGAGCT	960

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TTCATTGAAA TTGAAGAGGG CATTGAAGGT CTAATTCACA TTTCTGAAAT GTCTTGGGTG 1020
 AAAAATATTG TAGATCCTAG TGAAGTCGTA AATAAAGGCG ATGAAGTTGA AGCCATTGTT 1080
 CTATCTATTC AGAAGGACGA AGGAAAAATT TCTCTAGGAT TAAAGCAAAC AGAACGTAAT 1140
 CCTTGGGACA ATATCGAAGA AAAATATCCT ATAGGTCTCC ATGTCAATGC TGAAATCAAG 1200
 AACTTAACCA ATTACGGTGC TTTCGTGAA TTAGAACCAG GAATTGAGGG TCTGATTCAT 1260
 ATTTCTGACA TGAGTTGGAT TAAAAAAGTC TCTCACCCTT CAGAACTATT CAAAAAGGA 1320
 AATTCTGTAG AGGCTGTTAT TTTATCAGTA GACAAAGAAA GTAAAAAAT TACTTTAGGA 1380
 GTTAAGCAAT TAAGTTCTAA TCCTTGAAT GAAATTGAAG CTATGTTCCC TGCTGGCACA 1440
 GTAATTCAG GAGTTGTGAC TAAATCACT GCATTGGAG CCTTTGTTGA GCTACAAAAC 1500
 GGGATTGAAG GATTGATTCA CGTTTCAGAA CTTTCTGACA AGCCCTTTGC AAAAATTGAA 1560
 GATATTATCT CCATTGGAGA AAATGTTTCT GCAAAGTAA TTAAGCTAGA TCCAGATCAT 1620
 AAAAAAGTTT CTCTTTCTGT AAAAGAATAC TTAGCTGACA ATGCTTATGA TCAAGACTCT 1680
 AGGACTGAAT TAGATTCAA GGATTCTCAA GG 1712

INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1048 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Chlamydia pneumoniae

(B) STRAIN: YK-41

(vii) IMMEDIATE SOURCE:

(B) CLONE: 53-3S

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 236 to 1012

(C) IDENTIFICATION METHOD: P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TCAGTATCGG CGGAATTCGA ACCCCTTCGC GGCTCTTTCT GGAAGTCTAG AATCTTTACA 60

TCTCGAAGAG TTAAGTCAAG GATTATTCCC TTCTGCCCAA GAAGATGCCA ACTTCGCAAA 120

GGAGTTATCT TCAGTAGTAC ACGGATTAAA AAACCTAACC ACTGTAGTTA ATAAACAAAT 180

GGTTAAAGGC GCTGAGTAAA GCCCTTTGCA GAATCAAACC CCTTAGGATA CAAAC ATG 238

Met

1

TCT ATT TCA TCT TCT TCA GGA CCT GAC AAT CAA AAA AAT ATC ATG TCT 286

Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile Met Ser

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	5	10	15	
5	CAA GTT CTG ACA TCG ACA CCC CAG GGC GTG CCC CAA CAA GAT AAG CTG			334
	Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp Lys Leu			
10	20	25	30	
	TCT GGC AAC GAA ACG AAG CAA ATA CAG CAA ACA CGT CAG GGT AAA AAC			382
15	Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly Lys Asn			
	35	40	45	
20	ACT GAG ATG GAA AGC GAT GCC ACT ATT GCT GGT GCT TCT GGA AAA GAC			430
	Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly Lys Asp			
25	50	55	60	65
	AAA ACT TCC TCG ACT ACA AAA ACA GAA ACA GCT CCA CAA CAG GGA GTT			478
30	Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln Gly Val			
	70	75	80	
	GCT GCT GGG AAA GAA TCC TCA GAA AGT CAA AAG GCA GGT GCT GAT ACT			526
35	Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala Asp Thr			
	85	90	95	
40	GGA GTA TCA GGA GCG GCT GCT ACT ACA GCA TCA AAT ACT GCA ACA AAA			574
45	Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala Thr Lys			
	100	105	110	
	ATT GCT ATG CAG ACC TCT ATT GAA GAG GCG AGC AAA AGT ATG GAG TCT			622
50	Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met Glu Ser			
	115	120	125	
55				

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ACC TTA GAG TCA CTT CAA AGC CTC AGT GCC GCG CAA ATG AAA GAA GTC 670

5 Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys Glu Val
130 135 140 145

10 GAA GCG GTT GTT GTT GCT GCC CTC TCA GGG AAA AGT TCG GGT TCC GCA 718

Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly Ser Ala
15 150 155 160

AAA TTG GAA ACA CCT GAG CTC CCC AAG CCC GGG GTG ACA CCA AGA TCA 766

20 Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro Arg Ser
165 170 175

GAG GTT ATC GAA ATC GGA CTC GCG CTT GCT AAA GCA ATT CAG ACA TTG 814

25 Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln Thr Leu
180 185 190

30 GGA GAA GCC ACA AAA TCT GCC TTA TCT AAC TAT GCA AGT ACA CAA GCA 862

35 Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr Gln Ala
195 200 205

CAA GCA GAC CAA ACA AAT AAA CTA GGT CTA GAA AAG CAA GCG ATA AAA 910

40 Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala Ile Lys
210 215 220 225

45 ATC GAT AAA GAA CGA GAA GAA TAC CAA GAG ATG AAG GCT GCC GAA CAG 958

Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala Glu Gln
50 230 235 240

AAG TCT AAA GAT CTC GAA GGA ACA ATG GAT ACT GTC AAT ACT GTG ATG 1006

55

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Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr Val Met

245

250

255

5

ATC GCG AAGGGGTTTCG AATTCCAGCT GAGCGCCGGT CGCTAC

1048

10

Ile Ala

259

15

INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH:5702 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

25

(ii) MOLECULE TYPE: Other nucleic acid; Plasmid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

30

ATCGATGTTA ACAGATCTAA GCTTAACTAA CTAAGTCCGG AAAAGGAGGA ACTTCCATGA 60

35

TCAGTCTGAT TCGGGCGTTA GCGGTAGATC GCGTTATCGG CATGGAAAAC GCCATGCCGT 120

GGAACCTGCC TGCCGATCTC GCCTGGTTTA AACGCAACAC CTAAATATAA CCCGTGATTA 180

40

TGGGCCGCCA TACCTGGGAA TCAATCGGTC GTCCGTGACC AGGACGCAAA AATATTATCC 240

45

TCAGCAGTCA ACCGGGTACG GACGATCGCG TAACGTGGGT GAAGTCGGTG GATGAAGCCA 300

TCGCGGCGTG TGGTGACGTA CCAGAAATCA TGGTGATTGG CGGCGGTCGC GTTATGAAC 360

50

AGTTCTTGCC AAAAGCGCAA AAAGTGTATC TGACGCATAT CGACGCAGAA GTGGAAGGCG 420

55

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ACACCCATTT CCCGATTAC GAGCCGGATG ACTGGGAATC GGTATTCAGC GAATTCCACG 480

5 ATGCTGATGC GCAGAACTCT CACAGCTATG AGTTCGAAAT TCTGGAGCGG CGGATCCAAT 540

10 TCGAACCCCT TCGCGGCTCT TTCTGGAAC TTAGAATCTT TACATCTCGA AGAGTTAACT 600

CAAGGATTAT TCCCTTCTGC CCAAGAAGAT GCCAACTTCG CAAAGGAGTT ATCTTCAGTA 660

15 GTACACGGAT TAAAAACCT AACCACTGTA GTTAATAAAC AAATGGTTAA AGGCGCTGAG 720

20 TAAAGCCCTT TGCAGAAATCA AACCCCTTAG GATACAAACA TGTCTATTTT ATCTTCTTCA 780

25 GGACCTGACA ATCAAAAAA TATCATGTCT CAAGTTCTGA CATCGACACC CCAGGGCGTG 840

CCCCAACAAG ATAAGCTGTC TGGCAACGAA ACGAAGCAA TACAGCAAAC ACGTCAGGGT 900

30 AAAAAGCTG AGATGGAAAG CGATGCCACT ATTGCTGGTG CTCTGGAAA AGACAAAAT 960

35 TCCTCGACTA CAAAAACAGA AACAGCTCCA CAACAGGGAG TTGCTGCTGG GAAAGAATCC 1020

TCAGAAAGTC AAAAGGCAGG TGCTGATACT GGAGTATCAG GAGCGGCTGC TACTACAGCA 1080

40 TCAAATACTG CAACAAAAT TGCTATGCAG ACCTCTATTG AAGAGGCGAG CAAAAGTATG 1140

45 GAGTCTACCT TAGAGTCACT TCAAAGCCTC AGTGCCGCGC AAATGAAAGA AGTCGAAGCG 1200

50 GTTGTGTTG CTGCCCTCTC AGGGAAAAGT TCGGTTCCG CAAAATTGGA AACACCTGAG 1260

55

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CTCCCCAAGC CCGGGGTGAC ACCAAGATCA GAGGTTATCG AAATCGGACT CGCGCTTGCT 1320

5 AAAGCAATTC AGACATTGGG AGAAGCCACA AAATCTGCCT TATCTAACTA TGCAAGTACA 1380

10 CAAGCACAAG CAGACCAAAC AAATAAACTA GGTCTAGAAA AGCAAGCGAT AAAAATCGAT 1440

AAAGAACGAG AAGAATACCA AGAGATGAAG GCTGCCGAAC AGAAGTCTAA AGATCTCGAA 1500

15 GGAACAATGG ATACTGTCAA TACTGTGATG ATCGCGAAGG GGTTCGAATT GCCATGGGGG 1560

20 CCCTTAATTA ATTAAGTCGA GAGATCCAGA TCTAATCGAT GATCCTCTAC GCCGGACGCA 1620

25 TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG CGCCTATATC GCCGACATCA 1680

CCGATGGGGA AGATCGGGCT CGCCACTTCG GGTCATGAG CGCTTGTTTC GGCGTGGGTA 1740

30 TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC TCCTTGCATG CACCATTCTT 1800

35 TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC TGCTTCCTAA TGCAGGAGTC 1860

GCATAAGGGA GAGCGTCGAC CGATGCCCTT GAGAGCCTTC AACCCAGTCA GTCCTTCCG 1920

40 GTGGGCGCGG GGCATGACTA TCGTCGCCGC ACTTATGACT GTCTTCTTTA TCATGCAACT 1980

45 CGTAGGACAG GTGCCGGCAG CGCTCTGGGT CATTTTCGGC GAGGACCGCT TTCGCTGGAG 2040

50 CGCGACGATG ATCGGCCTGT CGCTTGCGGT ATTCGGAATC TTGCACGCC CCGCTCAAGC 2100

CTTCGTCACT GGTCCCGCCA CCAAACGTTT CGGCGAGAAG CAGGCCATTA TCGCCGGCAT 2160

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GGCGGCCGAC GCGCTGGGCT ACGTCTTGCT GGCCTTCGCG ACGCGAGGCT GGATGGCCTT 2220

5 CCCCATTATG ATTCTTCTCG CTTCGGGCGG CATCGGGATG CCCGCGTTGC AGGCCATGCT 2280

10 GTCCAGGCAG GTAGATGACG ACCATCAGGG ACAGCTTCAA GGATCGCTCG CGGCTCTTAC 2340

CAGCCTAACT TCGATCACTG GACCGCTGAT CGTCACGGCG ATTTATGCCG CCTCGGCGAG 2400

15 CACATGGAAC GGGTTGGCAT GGATTGTAGG CGCCGCCCTA TACCTTGTCT GCCTCCCCGC 2460

20 GTTGCGTCGC GGTGCATGGA GCCGGGCCAC CTCGACCTGA ATGGAAGCCG GCGGCACCTC 2520

GCTAACGGAT TCACCACTCC AAGAATTGGA GCCAATCAAT TCTTGCGGAG AACTGTGAAT 2580

25 GCGCAAACCA ACCCTTGGCA GAACATATCC ATCGCGTCCG CCATCTCCAG CAGCCGCACG 2640

30 CGGCGCATCT CGGGCAGCGT TGGGTCTTGG CCACGGGTGC GCATGATCGT GCTCCTGTCTG 2700

35 TTGAGGACCC GGCTAGGCTG GCGGGGTGCG CTTACTGGTT AGCAGAATGA ATCACCGATA 2760

CGCGAGCGAA CGTGAAGCGA CTGCTGCTGC AAAACGTCTG CGACCTGAGC AACAAATGA 2820

40 ATGGTCTTCG GTTCCGTGT TTCGTAAAGT CTGGAACGC GGAAGTCAGC GCCCTGCACC 2880

45 ATTATGTTCC GGATCTGCAT CGCAGGATGC TGCTGGCTAC CCTGTGGAAC ACCTACATCT 2940

50 GTATTAACGA AGCGCTGGCA TTGACCCTGA GTGATTTTTC TCTGGTCCCG CCGCATCCAT 3000

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ACCGCCAGTT GTTTACCCTC ACAACGTTCC AGTAACCGGG CATGTTTCATC ATCAGTAACC 3060

5 CGTATCGTGA GCATCCTCTC TCGTTTCATC GGTATCATTA CCCCATGAA CAGAAATTC 3120

10 CCCCTTACAC GGAGGCATCA AGTGACCAAA CAGGAAAAAA CCGCCCTTAA CATGGCCCG 3180

CTTTATCAGA AGCCAGACAT TAACGCTTCT GGAGAACTC AACGAGCTGG ACGCGGATG 3240

15 AACAGGCAGA CATCTGTGAA TCGCTTCACG ACCACGCTGA TGAGCTTTAC CGCAGCTGC 3300

20 CTCGCGCGTT TCGGTGATGA CGGTGAAAAC CTCTGACACA TGCAGCTCCC GGAGACGGT 3360

25 CACAGCTTGT CTGTAAGCGG ATGCCGGGAG CAGACAAGCC CGTCAGGGCG CGTCAGCGG 3420

GTGTTGGCGG GTGTCGGGGC GCAGCCATGA CCCAGTCACG TAGCGATAGC GGAGTGTAT 3480

30 ACTGGCTTAA CTATGCGGCA TCAGAGCAGA TTGTACTGAG AGTGCACCAT ATGCGGTGT 3540

35 GAAATACCGC ACAGATGCGT AAGGAGAAAA TACCGCATCA GCGCTCTTC CGCTTCCTC 3600

GCTCACTGAC TCGTGGGCT CGGTGTTTCG GCTGCGGCGA GCGGTATCAG CTCACTCAA 3660

40 AGGCGGTAAT ACGGTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCA 3720

45 AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAG 3780

50 GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACC 3840

CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG CGCTCTCCT 3900

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GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGC 3960
 5 GCTTTCTCAA TGCTCAGCT GTAGGTATCT CAGTTCGGTG TAGGTCGTTC GCTCCAAGC 4020
 10 TGGGCTGTGT GCACGAACCC CCCGTTACG CCGACCGCTG CGCCTTATCC GGTAACCTAT 4080
 CGTCTTGAGT CCAACCCGGT AAGACACGAC TTATCGCCAC TGGCAGCAGC CACTGGTAA 4140
 15 CAGGATTAGC AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTA 4200
 20 ACTACGGCTA CACTAGAAGG ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACC 4260
 25 TTCGAAAAA GAGTTGGTAG CTCTTGATCC GGCAAACAAA CCACCGCTGG TAGCGGTGG 4320
 TTTTTTGTG TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG AAGATCCTT 4380
 30 TGATCTTTTC TACGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTG 4440
 35 GTCATGAGAT TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTT 4500
 TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT GCTTAATCA 4560
 40 GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTTCATC CATAGTTGCC TGAATCCC 4620
 45 GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCAGTGC TGCAATGAT 4680
 50 ACCGCGAGAC CCACGCTCAC CGGCTCCAGA TTTATCAGCA ATAAACCAGC CAGCCGGAA 4740
 55

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	GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGT	4800
5	TGCCGGAAG CTAGAGTAAG TAGTTCGCCA GTTAATAGTT TGCACAACGT TGTTGCCAT	4860
10	TGCTGCAGGC ATCGTGGTGT CACGCTCGTC GTTGGTATG GCTTCATTCA GCTCCGGTT	4920
	CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCC	4980
15	TTCGGTCTC CGATCGTTGT CAGAAGTAAG TTGGCCGAG TGTATCACT CATGGTTAT	5040
20	GGCAGCACTG CATAATTCTC TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTG	5100
25	GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGATGCG GCGACCGAGT TGCTCTTGC	5160
	CCGGCGTCAA CACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GTCATCAT	5220
30	TGAAAAACGT TCTTCGGGGC GAAACTCTC AAGGATCTTA CCGCTGTTGA GATCCAGTT	5280
35	CGATGTAACC CACTCGTGCA CCCAACTGAT CTTCAGCATC TTTTACTTTC ACCAGCGTT	5340
	TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAA AGGAATAAG GCGACACG	5400
40	GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT TGAAGCATTT ATCAGGGTT	5460
45	ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA AAATAAACAA ATAGGGGTT	5520
50	CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGAC	5580
55	ATTAACCTAT AAAAATAGGC GTATCAGAG GCCCTTTCGT CTTCAAGAAT TAATTGTTA	5640

TCCGCTCACA ATTAATTCTT GACAATTAGT TAACTATTTG TTATAATGTA TTCATAAGC 5700

5 TT 5702

10 INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH:35

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

20 (ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GATCCAATTG CCATGGGGC CCTTAATTAA TTAAC 35

25

INFORMATION FOR SEQ ID NO: 12:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:35 base pairs

35 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TCGAGTTAAT TAATTAAGGG CCCCCATGGC AATTG 35

45

50

55

INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH:1954 base pairs

(B) TYPE: nucleic acid

10

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE:Genomic DNA

15

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Chlamydia pneumoniae

(B) STRAIN: YK-41

20

(vii) IMMEDIATE SOURCE:

(B) CLONE: 70-2S

(ix) FEATURE:

25

(A) NAME/KEY: -35 signal

(B) LOCATION:146 to 151

30

(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus sequence

(ix) FEATURE:

35

(A) NAME/KEY: -10 signal

(B) LOCATION:169 to 174

40

(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus sequence

(ix) FEATURE:

(A) NAME/KEY: RBS

45

(B) LOCATION:199 to 205

(C) IDENTIFICATION METHOD: by similarity with known sequence or to an established consensus sequence

50

(ix) FEATURE:

(A) NAME/KEY:CDS

55

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(B) LOCATION: 215 to 1927

(C) IDENTIFICATION METHOD: by similarity with known sequence or to an
established consensus sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

TTGACACCAG ACCAACTGGT AATGGTAGCG ACCGGCGCTC AGCTGGAATT CGAACCCCTT 60

CGCCTTATAC ATCTCTAGAA CGGAAGTATA GGATTTTACG ATTAATTCGA TTATATAGAA 120

CTAATCGTCT CCTGCAAGGG AGGTCTTGCC TTTTTTAAGG TTTATATTTA CACTGTCTTT 180

TTTGACTTTG TAGTTTTTAG GAGAATAACA ATAA ATG CCA AAA CAA GCT GAA TAT 235

Met Pro Lys Gln Ala Glu Tyr

ACT TGG GGA TCT AAA AAA ATT CTG GAC AAT ATA GAA TGC CTC ACA GAA 283

Thr Trp Gly Ser Lys Lys Ile Leu Asp Asn Ile Glu Cys Leu Thr Glu

GAC GTT GCC GAA TTT AAA GAT TTG CTT TAT ACG GCA CAC AGA ATT ACT 331

Asp Val Ala Glu Phe Lys Asp Leu Leu Tyr Thr Ala His Arg Ile Thr

TCG AGC GAA GAA GAA TCT GAT AAC GAA ATA CAG CCT GGC GCC ATC CTA 379

Ser Ser Glu Glu Glu Ser Asp Asn Glu Ile Gln Pro Gly Ala Ile Leu

AAA GGT ACC GTA GTT GAT ATT AAT AAA GAC TTT GTC GTA GTT GAT GTT 427

Lys Gly Thr Val Val Asp Ile Asn Lys Asp Phe Val Val Val Asp Val

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	60	65	70	
5	GGT CTG AAG TCT GAG GGA GTG ATC CCT ATG TCA GAG TTC ATA GAC TCT			475
	Gly Leu Lys Ser Glu Gly Val Ile Pro Met Ser Glu Phe Ile Asp Ser			
	75	80	85	
10	TCA GAA GGT TTA GTG CTT GGA GCT GAA GTA GAA GTC TAT CTC GAC CAA			523
	Ser Glu Gly Leu Val Leu Gly Ala Glu Val Glu Val Tyr Leu Asp Gln			
15	90	95	100	
	GCC GAA GAC GAA GAG GGC AAA GTT GTC CTT TCT AGA GAA AAA GCC ACA			571
20	Ala Glu Asp Glu Glu Gly Lys Val Val Leu Ser Arg Glu Lys Ala Thr			
	105	110	115	
25	CGA CAA CGT CAA TGG GAA TAC ATC TTA GCT CAT TGT GAA GAA GGT TCT			619
	Arg Gln Arg Gln Trp Glu Tyr Ile Leu Ala His Cys Glu Glu Gly Ser			
30	120	125	130	135
	ATT GTT AAA GGT CAA ATT ACA CGT AAA GTC AAA GGC GGC CTT ATT GTA			667
35	Ile Val Lys Gly Gln Ile Thr Arg Lys Val Lys Gly Gly Leu Ile Val			
	140	145	150	
40	GAT Ile Gly Met Glu Ala Phe Leu Pro Gly Ser Gln Ile Asp Asn Lys			715
	Asp ATT GGA ATG GAA GCC TTC CTA CCT GGA TCA CAA ATT GAC AAC AAG			
45	155	160	165	
	Lys ATC AAA AAT TTA GAT GAT TAT GTC GGA AAA GTT TGT GAA TTC AAA			763
50	AAA Ile Lys Asn Leu Asp Asp Tyr Val Gly Lys Val Cys Glu Phe Lys			
	170	175	180	
55				

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ATT TTA AAA ATT AAC GTT GAA CGT CGC AAT ATT GTT GTC TCA AGA AGA 811

5
Ile Leu Lys Ile Asn Val Glu Arg Arg Asn Ile Val Val Ser Arg Arg
185 190 195

10
GAA CTC TTA GAA GCT GAG AGA ATC TCT AAG AAA GCC GAA CTT ATT GAA 859

15
Glu Leu Leu Glu Ala Glu Arg Ile Ser Lys Lys Ala Glu Leu Ile Glu
200 205 210 215
CAA ATT TCT ATC GGA GAA TAC CGC AAA GGA GTT GTT AAA AAC ATT ACT 907

20
Gln Ile Ser Ile Gly Glu Tyr Arg Lys Gly Val Val Lys Asn Ile Thr
220 225 230
GAC TTT GGT GTA TTC TTA GAT CTC GAT GGT ATT GAC GGT CTT CTC CAC 955

25
Asp Phe Gly Val Phe Leu Asp Leu Asp Gly Ile Asp Gly Leu Leu His
235 240 245

30
ATT ACC GAT ATG ACC TGG AAG CGC ATA CGA CAT CCT TCC GAA ATG GTC 1003

35
Ile Thr Asp Met Thr Trp Lys Arg Ile Arg His Pro Ser Glu Met Val
250 255 260
GAA TTG AAT CAA GAG TTG GAA GTA ATT ATT TTA AGC GTA GAT AAA GAA 1051

40
Glu Leu Asn Gln Glu Leu Glu Val Ile Ile Leu Ser Val Asp Lys Glu
265 270 275

45
AAA GGA CGA GTT GCT CTA GGT CTC AAA CAA AAA GAG CAT AAT CCT TGG 1099

50
Lys Gly Arg Val Ala Leu Gly Leu Lys Gln Lys Glu His Asn Pro Trp
280 285 290 295
GAA GAT ATT GAG AAG AAA TAC CCT CCT GGA AAA CGA GTT CTT GGT AAA 1147

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	Glu Asp Ile Glu Lys Lys Tyr Pro Pro Gly Lys Arg Val Leu Gly Lys	
	300 305 310	
5	ATT GTG AAG CTT CTC CCC TAC GGA GCT TTC ATT GAA ATT GAA GAG GGC	1195
10	Ile Val Lys Leu Leu Pro Tyr Gly Ala Phe Ile Glu Ile Glu Glu Gly	
	315 320 325	
	ATT GAA GGT CTA ATT CAC ATT TCT GAA ATG TCT TGG GTG AAA AAT ATT	1243
15	Ile Glu Gly Leu Ile His Ile Ser Glu Met Ser Trp Val Lys Asn Ile	
	330 335 340	
20	GTA GAT CCT AGT GAA GTC GTA AAT AAA GGC GAT GAA GTT GAA GCC ATT	1291
25	Val Asp Pro Ser Glu Val Val Asn Lys Gly Asp Glu Val Glu Ala Ile	
	345 350 355	
	GTT CTA TCT ATT CAG AAG GAC GAA GGA AAA ATT TCT CTA GGA TTA AAG	1339
30	Val Leu Ser Ile Gln Lys Asp Glu Gly Lys Ile Ser Leu Gly Leu Lys	
	360 365 370 375	
35	CAA ACA GAA CGT AAT CCT TGG GAC AAT ATC GAA GAA AAA TAT CCT ATA	1387
40	Gln Thr Glu Arg Asn Pro Trp Asp Asn Ile Glu Glu Lys Tyr Pro Ile	
	380 385 390	
	GGT CTC CAT GTC AAT GCT GAA ATC AAG AAC TTA ACC AAT TAC GGT GCT	1435
45	Gly Leu His Val Asn Ala Glu Ile Lys Asn Leu Thr Asn Tyr Gly Ala	
	395 400 405	
50	TTC GTT GAA TTA GAA CCA GGA ATT GAG GGT CTG ATT CAT ATT TCT GAC	1483
55		

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Phe Val Glu Leu Glu Pro Gly Ile Glu Gly Leu Ile His Ile Ser Asp
410 415 420

5 ATG AGT TGG ATT AAA AAA GTC TCT CAC CCT TCA GAA CTA TTC AAA AAA 1531

Met Ser Trp Ile Lys Lys Val Ser His Pro Ser Glu Leu Phe Lys Lys
425 430 435

10 GGA AAT TCT GTA GAG GCT GTT ATT TTA TCA GTA GAC AAA GAA AGT AAA 1579

Gly Asn Ser Val Glu Ala Val Ile Leu Ser Val Asp Lys Glu Ser Lys
440 445 450 455

15 AAA ATT ACT TTA GGA GTT AAG CAA TTA AGT TCT AAT CCT TGG AAT GAA 1627

Lys Ile Thr Leu Gly Val Lys Gln Leu Ser Ser Asn Pro Trp Asn Glu
460 465 470

20 ATT GAA GCT ATG TTC CCT GCT GGC ACA GTA ATT TCA GGA GTT GTG ACT 1675

Ile Glu Ala Met Phe Pro Ala Gly Thr Val Ile Ser Gly Val Val Thr
475 480 485

30 AAA ATC ACT GCA TTT GGA GCC TTT GTT GAG CTA CAA AAC GGG ATT GAA 1723

Lys Ile Thr Ala Phe Gly Ala Phe Val Glu Leu Gln Asn Gly Ile Glu
490 495 500

35 GGA TTG ATT CAC GTT TCA GAA CTT TCT GAC AAG CCC TTT GCA AAA ATT 1771

Gly Leu Ile His Val Ser Glu Leu Ser Asp Lys Pro Phe Ala Lys Ile
505 510 515

40 GAA GAT ATT ATC TCC ATT GGA GAA AAT GTT TCT GCA AAA GTA ATT AAG 1919

Glu Asp Ile Ile Ser Ile Gly Glu Asn Val Ser Ala Lys Val Ile Lys

50

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520 525 530 535
 CTA GAT CCA GAT CAT AAA AAA GTT TCT CTT TCT GTA AAA GAA TAC TTA 1867
 5
 Leu Asp Pro Asp His Lys Lys Val Ser Leu Ser Val Lys Glu Tyr Leu
 540 545 550
 10 GCT GAC AAT GCT TAT GAT CAA GAC TCT AGG ACT GAA TTA GAT TTC AAG 1915
 Ala Asp Asn Ala Tyr Asp Gln Asp Ser Arg Thr Glu Leu Asp Phe Lys
 555 560 565
 15 GAT TCT CAA GGC GAA GGG GTT CGA ATT CCG CCG ATA CTG 1954
 20 Asp Ser Gln Gly Glu Gly Val Arg Ile Pro Pro Ile Leu
 570 575 580
 25

INFORMATION FOR SEQ ID NO: 14:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH:160 amino acids
 (B) TYPE: amino acid
 35 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
 40 Met Ile Ser Leu Ile Ala Ala Leu Ala Val Asp Arg Val Ile Gly Met
 1 5 10 15
 Glu Asn Ala Met Pro Trp Asn Leu Pro Ala Asp Leu Ala Trp Phe Lys
 45 20 25 30
 Arg Asn Thr Leu Asn Lys Pro Val Ile Met Gly Arg His Thr Trp Glu
 35 40 45
 50 Ser Ile Gly Arg Pro Leu Pro Gly Arg Lys Asn Ile Ile Leu Ser Ser
 50 55 60
 55

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Gln Pro Gly Thr Asp Asp Arg Val Thr Trp Val Lys Ser Val Asp Glu
65 70 75 80
5 Ala Ile Ala Ala Cys Gly Asp Val Pro Glu Ile Met Val Ile Gly Gly
85 90 95
10 Gly Arg Val Tyr Glu Gln Phe Leu Pro Lys Ala Gln Lys Leu Tyr Leu
100 105 110
Thr His Ile Asp Ala Glu Val Glu Gly Asp Thr His Phe Pro Asp Tyr
115 120 125
15 Glu Pro Asp Asp Trp Glu Ser Val Phe Ser Glu Phe His Asp Ala Asp
130 135 140
20 Ala Gln Asn Ser His Ser Tyr Glu Phe Glu Ile Leu Glu Arg Arg Ile
145 150 155 160

25 INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH:649 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: peptide

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Met Ile Ser Leu Ile Ala Ala Leu Ala Val Asp Arg Val Ile Gly Met

1 5 10 15
40 Glu Asn Ala Met Pro Trp Asn Leu Pro Ala Asp Leu Ala Trp Phe Lys

20 25 30
45 Arg Asn Thr Leu Asn Lys Pro Val Ile Met Gly Arg His Thr Trp Glu

35 40 45
Ser Ile Gly Arg Pro Leu Pro Gly Arg Lys Asn Ile Ile Leu Ser Ser

50 55 60
55 Gln Pro Gly Thr Asp Asp Arg Val Thr Trp Val Lys Ser Val Asp Glu

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	65	70	75	80
	Ala Ile Ala Ala Cys Gly Asp Val Pro Glu Ile Met Val Ile Gly Gly			
5		85	90	95
	Gly Arg Val Tyr Glu Gln Phe Leu Pro Lys Ala Gln Lys Leu Tyr Leu			
	100	105	110	
10	Thr His Ile Asp Ala Glu Val Glu Gly Asp Thr His Phe Pro Asp Tyr			
	115	120	125	
15	Glu Pro Asp Asp Trp Glu Ser Val Phe Ser Glu Phe His Asp Ala Asp			
	130	135	140	
	Ala Gln Asn Ser His Ser Tyr Glu Phe Glu Ile Leu Glu Arg Arg Ile			
20	145	150	155	160
	Leu Met Ser Ile Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile			
	165	170	175	
25	Met Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp			
	180	185	190	
	Lys Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly			
30	195	200	205	
	Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly			
35	210	215	220	
	Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln			
	225	230	235	240
40	Gly Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala			
	245	250	255	
	Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala			
45	260	265	270	
	Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met			
	275	280	285	
50	Glu Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys			
	290	295	300	

55

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Glu Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly
 305 310 315 320
 5 Ser Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro
 325 330 335
 Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln
 10 340 345 350
 Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr
 355 360 365
 15 Gln Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala
 370 375 380
 Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala
 20 385 390 395 400
 Glu Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr
 25 405 410 415
 Val Met Ile Ala Val Ser Val Ala Ile Thr Val Ile Ser Ile Val Ala
 420 425 430
 30 Ala Ile Phe Thr Cys Gly Ala Gly Leu Ala Gly Leu Ala Ala Gly Ala
 435 440 445
 Ala Val Gly Ala Ala Ala Ala Gly Gly Ala Ala Gly Ala Ala Ala Ala
 35 450 455 460
 Thr Thr Val Ala Thr Gln Ile Thr Val Gln Ala Val Val Gln Ala Val
 465 470 475 480
 40 Lys Gln Ala Val Ile Thr Ala Val Arg Gln Ala Ile Thr Ala Ala Ile
 485 490 495
 Lys Ala Ala Val Lys Ser Gly Ile Lys Ala Phe Ile Lys Thr Leu Val
 45 500 505 510
 Lys Ala Ile Ala Lys Ala Ile Ser Lys Gly Ile Ser Lys Val Phe Ala
 50 515 520 525
 Lys Gly Thr Gln Met Ile Ala Lys Asn Phe Pro Lys Leu Ser Lys Val

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530 535 540
 Ile Ser Ser Leu Thr Ser Lys Trp Val Thr Val Gly Val Gly Val Val
 5 545 550 555 560
 Val Ala Ala Pro Ala Leu Gly Lys Gly Ile Met Gln Met Gln Leu Ser
 565 570 575
 10 Glu Met Gln Gln Asn Val Ala Gln Phe Gln Lys Glu Val Gly Lys Leu
 580 585 590
 15 Gln Ala Ala Ala Asp Met Ile Ser Met Phe Thr Gln Phe Trp Gln Gln
 595 600 605
 Ala Ser Lys Ile Ala Ser Lys Gln Thr Gly Glu Ser Asn Glu Met Thr
 20 610 615 620
 Gln Lys Ala Thr Lys Leu Gly Ala Gln Ile Leu Lys Ala Tyr Ala Ala
 625 630 635 640
 25 Ile Ser Gly Ala Ile Ala Gly Ala Ala
 645 649

30

INFORMATION FOR SEQ ID NO: 16:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:432 amino acids

(B) TYPE: amino acid

40 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Met Ile Ser Leu Ile Ala Ala Leu Ala Val Asp Arg Val Ile Gly Met
 45 1 5 10 15
 Glu Asn Ala Met Pro Trp Asn Leu Pro Ala Asp Leu Ala Trp Phe Lys
 20 25 30
 50 Arg Asn Thr Leu Asn Lys Pro Val Ile Met Gly Arg His Thr Trp Glu
 35 40 45

55

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Ser Ile Gly Arg Pro Leu Pro Gly Arg Lys Asn Ile Ile Leu Ser Ser
50 55 60
5 Gln Pro Gly Thr Asp Asp Arg Val Thr Trp Val Lys Ser Val Asp Glu
65 70 75 80
Ala Ile Ala Ala Cys Gly Asp Val Pro Glu Ile Met Val Ile Gly Gly
10 85 90 95
Gly Arg Val Tyr Glu Gln Phe Leu Pro Lys Ala Gln Lys Leu Tyr Leu
100 105 110
15 Thr His Ile Asp Ala Glu Val Glu Gly Asp Thr His Phe Pro Asp Tyr
115 120 125
20 Glu Pro Asp Asp Trp Glu Ser Val Phe Ser Glu Phe His Asp Ala Asp
130 135 140
Ala Gln Asn Ser His Ser Tyr Glu Phe Glu Ile Leu Glu Arg Arg Ile
25 145 150 155 160
Leu Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile
165 170 175
30 Met Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp
180 185 190
Lys Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly
35 195 200 205
Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly
210 215 220
40 Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln
225 230 235 240
45 Gly Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala
245 250 255
Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala
50 260 265 270
Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met
55

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275 280 285
 5 Glu Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys
 290 295 300
 Glu Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly
 10 305 310 315 320
 Ser Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro
 325 330 335
 15 Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln
 340 345 350
 Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr
 20 355 360 365
 Gln Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala
 370 375 380
 25 Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala
 385 390 395 400
 Glu Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr
 30 405 410 415
 Val Met Ile Ala Lys Gly Phe Glu Leu Pro Trp Gly Pro Leu Ile Asn
 420 425 430 432
 35

40 INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1947 base pairs

45 (B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ATG ATC AGT CTG ATT GCG GCG TTA GCG GTA GAT CGC GTT ATC GGC ATG

48

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	Met Ile Ser Leu Ile Ala Ala Leu Ala Val Asp Arg Val Ile Gly Met	
	1 5 10 15	
5	GAA AAC GCC ATG CCG TGG AAC CTG CCT GCC GAT CTC GCC TGG TTT AAA	96
	Glu Asn Ala Met Pro Trp Asn Leu Pro Ala Asp Leu Ala Trp Phe Lys	
10	20 25 30	
	CGC AAC ACC TTA AAT AAA CCC GTG ATT ATG GGC CGC CAT ACC TGG GAA	144
15	Arg Asn Thr Leu Asn Lys Pro Val Ile Met Gly Arg His Thr Trp Glu	
	35 40 45	
20	TCA ATC GGT CGT CCG TTG CCA GGA CGC AAA AAT ATT ATC CTC AGC AGT	192
	Ser Ile Gly Arg Pro Leu Pro Gly Arg Lys Asn Ile Ile Leu Ser Ser	
25	50 55 60	
	CAA CCG GGT ACG GAC GAT CGC GTA ACG TGG GTG AAG TCG GTG GAT GAA	240
30	Gln Pro Gly Thr Asp Asp Arg Val Thr Trp Val Lys Ser Val Asp Glu	
	65 70 75 80	
35	GCC ATC GCG GCG TGT GGT GAC GTA CCA GAA ATC ATG GTG ATT GGC GGC	288
	Ala Ile Ala Ala Cys Gly Asp Val Pro Glu Ile Met Val Ile Gly Gly	
40	85 90 95	
	GGT CGC GTT TAT GAA CAG TTC TTG CCA AAA GCG CAA AAA CTG TAT CTG	336
45	Gly Arg Val Tyr Glu Gln Phe Leu Pro Lys Ala Gln Lys Leu Tyr Leu	
	100 105 110	
50	ACG CAT ATC GAC GCA GAA GTG GAA GGC GAC ACC CAT TTC CCG GAT TAC	384
55		

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Thr His Ile Asp Ala Glu Val Glu Gly Asp Thr His Phe Pro Asp Tyr
115 120 125
5 GAG CCG GAT GAC TGG GAA TCG GTA TTC AGC GAA TTC CAC GAT GCT GAT 432

Glu Pro Asp Asp Trp Glu Ser Val Phe Ser Glu Phe His Asp Ala Asp
130 135 140
10 GCG CAG AAC TCT CAC AGC TAT GAG TTC GAA ATT CTG GAG CGG CGG ATC 480

Ala Gln Asn Ser His Ser Tyr Glu Phe Glu Ile Leu Glu Arg Arg Ile
145 150 155 160
20 CTG ATG TCT ATT TCA TCT TCT TCA GGA CCT GAC AAT CAA AAA AAT ATC 528

Leu Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile
165 170 175
25 ATG TCT CAA GTT CTG ACA TCG ACA CCC CAG GGC GTG CCC CAA CAA GAT 576

Met Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp
180 185 190
30 AAG CTG TCT GGC AAC GAA ACG AAG CAA ATA CAG CAA ACA CGT CAG GGT 624

Lys Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly
195 200 205
40 AAA AAC ACT GAG ATG GAA AGC GAT GCC ACT ATT GCT GGT GCT TCT GGA 672

Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly
210 215 220
45 AAA GAC AAA ACT TCC TCG ACT ACA AAA ACA GAA ACA GCT CCA CAA CAG 720

Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln
50
55

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225 230 235 240
 GGA GTT GCT GCT GGG AAA GAA TCC TCA GAA AGT CAA AAG GCA GGT GCT 768
 5
 Gly Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala
 245 250 255
 10 GAT ACT GGA GTA TCA GGA GCG GCT GCT ACT ACA GCA TCA AAT ACT GCA 816
 Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala
 15 260 265 270
 ACA AAA ATT GCT ATG CAG ACC TCT ATT GAA GAG GCG AGC AAA AGT ATG 864
 20 Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met
 275 280 285
 25 GAG TCT ACC TTA GAG TCA CTT CAA AGC CTC AGT GCC GCG CAA ATG AAA 912
 Glu Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys
 30 290 295 300
 GAA GTC GAA GCG GTT GTT GTT GCT GCC CTC TCA GGG AAA AGT TCG GGT 960
 35 Glu Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly
 305 310 315 320
 40 TCC GCA AAA TTG GAA ACA CCT GAG CTC CCC AAG CCC GGG GTG ACA CCA 1008
 Ser Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro
 45 325 330 335
 AGA TCA GAG GTT ATC GAA ATC GGA CTC GCG CTT GCT AAA GCA ATT CAG 1056
 50 Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln
 340 345 350
 55

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ACA TTG GGA GAA GCC ACA AAA TCT GCC TTA TCT AAC TAT GCA AGT ACA 1104

5 Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr
355 360 365

10 CAA GCA CAA GCA GAC CAA ACA AAT AAA CTA GGT CTA GAA AAG CAA GCG 1152

Gln Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala
15 370 375 380

ATA AAA ATC GAT AAA GAA CGA GAA GAA TAC CAA GAG ATG AAG GCT GCC 1200

20 Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala
385 390 395 400

GAA CAG AAG TCT AAA GAT CTC GAA GGA ACA ATG GAT ACT GTC AAT ACT 1248

25 Glu Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr
405 410 415

30 GTG ATG ATC GCG GTT TCT GTT GCC ATT ACA GTT ATT TCT ATT GTT GCT 1296

35 Val Met Ile Ala Val Ser Val Ala Ile Thr Val Ile Ser Ile Val Ala
420 425 430

GCT ATT TTT ACA TGC GGA GCT GGA CTC GCT GGA CTC GCT GCG GGA GCT 1344

40 Ala Ile Phe Thr Cys Gly Ala Gly Leu Ala Gly Leu Ala Ala Gly Ala
435 440 445

45 GCT GTA GGT GCA GCG GCA GCT GGA GGT GCA GCA GGA GCT GCT GCC GCA 1392

Ala Val Gly Ala Ala Ala Ala Gly Gly Ala Ala Gly Ala Ala Ala Ala
50 450 455 460

ACC ACG GTA GCA ACA CAA ATT ACA GTT CAA GCT GTT GTC CAA GCG GTG 1440

55

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Thr Thr Val Ala Thr Gln Ile Thr Val Gln Ala Val Val Gln Ala Val
465 470 475 480
5 AAA CAA GCT GTT ATC ACA GCT GTC AGA CAA GCG ATC ACC GCG GCT ATA 1488

Lys Gln Ala Val Ile Thr Ala Val Arg Gln Ala Ile Thr Ala Ala Ile
10 485 490 495
AAA GCG GCT GTC AAA TCT GGA ATA AAA GCA TTT ATC AAA ACT TTA GTC 1536

Lys Ala Ala Val Lys Ser Gly Ile Lys Ala Phe Ile Lys Thr Leu Val
15 500 505 510
20 AAA GCG ATT GCC AAA GCC ATT TCT AAA GGA ATC TCT AAG GTT TTC GCT 1584

Lys Ala Ile Ala Lys Ala Ile Ser Lys Gly Ile Ser Lys Val Phe Ala
25 515 520 525
AAG GGA ACT CAA ATG ATT GCG AAG AAC TTC CCC AAG CTC TCG AAA GTC 1632

Lys Gly Thr Gln Met Ile Ala Lys Asn Phe Pro Lys Leu Ser Lys Val
30 530 535 540
35 ATC TCG TCT CTT ACC AGT AAA TGG GTC ACG GTT GGG GTT GGG GTT GTA 1680

Ile Ser Ser Leu Thr Ser Lys Trp Val Thr Val Gly Val Gly Val Val
40 545 550 555 560
GTT GCG GCG CCT GCT CTC GGT AAA GGG ATT ATG CAA ATG CAG CTC TCG 1728

Val Ala Ala Pro Ala Leu Gly Lys Gly Ile Met Gln Met Gln Leu Ser
45 565 570 575
50 GAG ATG CAA CAA AAC GTC GCT CAA TTT CAG AAA GAA GTC GGA AAA CTG 1776

55

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Glu Met Gln Gln Asn Val Ala Gln Phe Gln Lys Glu Val Gly Lys Leu
580 585 590
5 CAG GCT GCG GCT GAT ATG ATT TCT ATG TTC ACT CAA TTT TGG CAA CAG 1824
Gln Ala Ala Ala Asp Met Ile Ser Met Phe Thr Gln Phe Trp Gln Gln
595 600 605
10 GCA AGT AAA ATT GCC TCA AAA CAA ACA GGC GAG TCT AAT GAA ATG ACT 1872
Ala Ser Lys Ile Ala Ser Lys Gln Thr Gly Glu Ser Asn Glu Met Thr
610 615 620
15 CAA AAA GCT ACC AAG CTG GGC GCT CAA ATC CTT AAA GCG TAT GCC GCA 1920
Gln Lys Ala Thr Lys Leu Gly Ala Gln Ile Leu Lys Ala Tyr Ala Ala
625 630 635 640
20 ATC AGC GGA GCC ATC GCT GGC GCA GCA 1947
Ile Ser Gly Ala Ile Ala Gly Ala Ala
645 649
25
30
35

INFORMATION FOR SEQ ID NO: 18:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:1296 base pairs

(B) TYPE: nucleic acid

45 (C) STRANDEDNESS: double

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

50 ATG ATC AGT CTG ATT GCG GCG TTA GCG GTA GAT CGC GTT ATC GGC ATG 48
55

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Met Ile Ser Leu Ile Ala Ala Leu Ala Val Asp Arg Val Ile Gly Met
1 5 10 15
5 GAA AAC GCC ATG CCG TGG AAC CTG CCT GCC GAT CTC GCC TGG TTT AAA 96

Glu Asn Ala Met Pro Trp Asn Leu Pro Ala Asp Leu Ala Trp Phe Lys
10 20 25 30
CGC AAC ACC TTA AAT AAA CCC GTG ATT ATG GGC CGC CAT ACC TGG GAA 144

Arg Asn Thr Leu Asn Lys Pro Val Ile Met Gly Arg His Thr Trp Glu
15 35 40 45
20 TCA ATC GGT CGT CCG TTG CCA GGA CGC AAA AAT ATT ATC CTC AGC AGT 192

Ser Ile Gly Arg Pro Leu Pro Gly Arg Lys Asn Ile Ile Leu Ser Ser
25 50 55 60
CAA CCG GGT ACG GAC GAT CGC GTA ACG TGG GTG AAG TCG GTG GAT GAA 240

Gln Pro Gly Thr Asp Asp Arg Val Thr Trp Val Lys Ser Val Asp Glu
30 65 70 75 80
35 GCC ATC GCG GCG TGT GGT GAC GTA CCA GAA ATC ATG GTG ATT GGC GGC 288

Ala Ile Ala Ala Cys Gly Asp Val Pro Glu Ile Met Val Ile Gly Gly
40 85 90 95
GGT CGC GTT TAT GAA CAG TTC TTG CCA AAA GCG CAA AAA CTG TAT CTG 336

Gly Arg Val Tyr Glu Gln Phe Leu Pro Lys Ala Gln Lys Leu Tyr Leu
45 100 105 110
ACG CAT ATC GAC GCA GAA GTG GAA GGC GAC ACC CAT TTC CCG GAT TAC 384

Thr His Ile Asp Ala Glu Val Glu Gly Asp Thr His Phe Pro Asp Tyr
50
55

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	115	120	125	
5	GAG CCG GAT GAC TGG GAA TCG GTA TTC AGC GAA TTC CAC GAT GCT GAT			432
	Glu Pro Asp Asp Trp Glu Ser Val Phe Ser Glu Phe His Asp Ala Asp			
10	130	135	140	
	GCG CAG AAC TCT CAC AGC TAT GAG TTC GAA ATT CTG GAG CGG CGG ATC			480
	Ala Gln Asn Ser His Ser Tyr Glu Phe Glu Ile Leu Glu Arg Arg Ile			
15	145	150	155	160
	CTG ATG TCT ATT TCA TCT TCT TCA GGA CCT GAC AAT CAA AAA AAT ATC			528
20				
	Leu Met Ser Ile Ser Ser Ser Ser Gly Pro Asp Asn Gln Lys Asn Ile			
	165	170	175	
25	ATG TCT CAA GTT CTG ACA TCG ACA CCC CAG GGC GTG CCC CAA CAA GAT			576
	Met Ser Gln Val Leu Thr Ser Thr Pro Gln Gly Val Pro Gln Gln Asp			
30	180	185	190	
	AAG CTG TCT GGC AAC GAA ACG AAG CAA ATA CAG CAA ACA CGT CAG GGT			624
35				
	Lys Leu Ser Gly Asn Glu Thr Lys Gln Ile Gln Gln Thr Arg Gln Gly			
	195	200	205	
40	AAA AAC ACT GAG ATG GAA AGC GAT GCC ACT ATT GCT GGT GCT TCT GGA			672
	Lys Asn Thr Glu Met Glu Ser Asp Ala Thr Ile Ala Gly Ala Ser Gly			
45	210	215	220	
	AAA GAC AAA ACT TCC TCG ACT ACA AAA ACA GAA ACA GCT CCA CAA CAG			720
50				
	Lys Asp Lys Thr Ser Ser Thr Thr Lys Thr Glu Thr Ala Pro Gln Gln			
	225	230	235	240
55				

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	GGA GTT GCT GCT GGG AAA GAA TCC TCA GAA AGT CAA AAG GCA GGT GCT	768
5	Gly Val Ala Ala Gly Lys Glu Ser Ser Glu Ser Gln Lys Ala Gly Ala	
	245 250 255	
10	GAT ACT GGA GTA TCA GGA GCG GCT GCT ACT ACA GCA TCA AAT ACT GCA	816
	Asp Thr Gly Val Ser Gly Ala Ala Ala Thr Thr Ala Ser Asn Thr Ala	
15	260 265 270	
	ACA AAA ATT GCT ATG CAG ACC TCT ATT GAA GAG GCG AGC AAA AGT ATG	864
20	Thr Lys Ile Ala Met Gln Thr Ser Ile Glu Glu Ala Ser Lys Ser Met	
	275 280 285	
25	GAG TCT ACC TTA GAG TCA CTT CAA AGC CTC AGT GCC GCG CAA ATG AAA	912
	Glu Ser Thr Leu Glu Ser Leu Gln Ser Leu Ser Ala Ala Gln Met Lys	
30	290 295 300	
	GAA GTC GAA GCG GTT GTT GTT GCT GCC CTC TCA GGG AAA AGT TCG GGT	960
35	Glu Val Glu Ala Val Val Val Ala Ala Leu Ser Gly Lys Ser Ser Gly	
	305 310 315 320	
40	TCC GCA AAA TTG GAA ACA CCT GAG CTC CCC AAG CCC GGG GTG ACA CCA	1008
	Ser Ala Lys Leu Glu Thr Pro Glu Leu Pro Lys Pro Gly Val Thr Pro	
45	325 330 335	
	AGA TCA GAG GTT ATC GAA ATC GGA CTC GCG CTT GCT AAA GCA ATT CAG	1056
50	Arg Ser Glu Val Ile Glu Ile Gly Leu Ala Leu Ala Lys Ala Ile Gln	
	340 345 350	
55	ACA TTG GGA GAA GCC ACA AAA TCT GCC TTA TCT AAC TAT GCA AGT ACA	1104

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Thr Leu Gly Glu Ala Thr Lys Ser Ala Leu Ser Asn Tyr Ala Ser Thr
355 360 365
5 CAA GCA CAA GCA GAC CAA ACA AAT AAA CTA GGT CTA GAA AAG CAA GCG 1152
Gln Ala Gln Ala Asp Gln Thr Asn Lys Leu Gly Leu Glu Lys Gln Ala
10 370 375 380
ATA AAA ATC GAT AAA GAA CGA GAA GAA TAC CAA GAG ATG AAG GCT GCC 1200
15 Ile Lys Ile Asp Lys Glu Arg Glu Glu Tyr Gln Glu Met Lys Ala Ala
385 390 395 400
20 GAA CAG AAG TCT AAA GAT CTC GAA GGA ACA ATG GAT ACT GTC AAT ACT 1248
Glu Gln Lys Ser Lys Asp Leu Glu Gly Thr Met Asp Thr Val Asn Thr
25 405 410 415
GTG ATG ATC GCG AAG GGG TTC GAA TTG CCA TGG GGG CCC TTA ATT AAT 1296
30 Val Met Ile Ala Lys Gly Phe Glu Leu Pro Trp Gly Pro Leu Ile Asn
420 425 430 432
35

INFORMATION FOR SEQ ID NO: 19:

40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 base pairs

(B) TYPE: nucleic acid

45 (C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

50 AGCTGTCTGG CAACGAAACG 20

55

INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GCAGCAACAA CAACCGCTTC

20

INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:29 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GATCCTGATG TCTATTTTCAT CTTCTTCAG

29

INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:28 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GTCCTGAAGA AGATGAAATA GACATCAG

28

5

INFORMATION FOR SEQ ID NO: 23:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 base pairs

(B) TYPE: nucleic acid

15

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

20

AATTGCCATG GGGGCCCTTA ATTAATTAAC

30

25

INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:30 base pairs

30

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

35

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

TCGAGTTAAT TAATTAAGGG CCCCCATGGC

30

40

INFORMATION FOR SEQ ID NO: 25:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:5438 base pairs

(B) TYPE: nucleic acid

50

(C) STRANDEDNESS: double

(ii) MOLECULE TYPE: Other nucleic acid; Plasmid

55

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

5	ATCGATGTTA ACAGATCTAA GCTTAACTAA CTAAGTCCGG AAAAGGAGGA ACTTCCATGA	60
	TCAGTCTGAT TCGGGCGTTA GCGGTAGATC GCGTTATCGG CATGGAAAAC GCCATGCCGT	120
10	GGAACCTGCC TGCCGATCTC GCCTGGTTTA AACGCAACAC CTTAAATAAA CCCGTGATTA	180
	TGGGCCGCCA TACCTGGGAA TCAATCGGTC GTCCGTGCC AGGACGCAA AATATTATCC	240
15	TCAGCAGTCA ACCGGGTACG GACGATCGCG TAACGTGGGT GAAGTCGGTG GATGAAGCCA	300
20	TCGCGGCGTG TGGTGACGTA CCAGAAATCA TGGTGATTGG CGGCGGTCGC GTTTATGAAC	360
	AGTTCTTGCC AAAAGCGCAA AACTGTATC TGACGCATAT CGACGCAGAA GTGGAAGGCG	420
25	ACACCCATTT CCCGGATTAC GAGCCGGATG ACTGGGAATC GGTATTCAGC GAATTCACG	480
30	ATGCTGATGC GCAGAACTCT CACAGCTATG AGTTCGAAAT TCTGGAGCGG CGGATCCTGA	540
35	TGTCTATTTC ATCTTCTTCA GGACCTGACA ATCAAAAAA TATCATGTCT CAAGTTCTGA	600
	CATCGACACC CCAGGGCGTG CCCCAACAAG ATAAGCTGTC TGGCAACGAA ACGAAGCAA	660
40	TACAGCAAAC ACGTCAGGT AAAAAGCTG AGATGGAAAG CGATGCCACT ATTGCTGGTG	720
45	CTTCTGGAAA AGACAAAAC TCCTCGACTA CAAAAACAGA AACAGCTCCA CAACAGGGAG	780
50	TTGCTGCTGG GAAAGAATCC TCAGAAAGTC AAAAGGCAGG TGCTGATACT GGAGTATCAG	840
55		

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GAGCGGCTGC TACTACAGCA TCAAATACTG CAACAAAAAT TGCTATGCAG ACCTCTATTG 900

5 AAGAGGCGAG CAAAAGTATG GAGTCTACCT TAGAGTCACT TCAAAGCCTC AGTGCCGCGC 960

10 AAATGAAAGA AGTCGAAGCG GTTGTGTGTG CTGCCCTCTC AGGGAAAAGT TCGGGTTCCG 1020

CAAATTTGGA AACACCTGAG CTCCCCAAGC CCGGGGTGAC ACCAAGATCA GAGGTTATCG 1080

15 AAATCGGACT CGCGCTTGCT AAAGCAATTC AGACATTGGG AGAAGCCACA AAATCTGCCT 1140

20 TATCTAACTA TGCAAGTACA CAAGCACAAAG CAGACCAAAC AAATAAACTA GGTCTAGAAA 1200

AGCAAGCGAT AAAAATCGAT AAAGAACGAG AAGAATACCA AGAGATGAAG GCTGCCGAAC 1260

25 AGAAGTCTAA AGATCTCGAA GGAACAATGG ATACTGTCAA TACTGTGATG ATCGCGAAGG 1320

30 GGTTCGAATT GCCATGGGGG CCCTTAATTA ATTAACTCGA GAGATCCAGA TCTAATCGAT 1380

35 GATCCTCTAC GCCGACGCA TCGTGGCCGG CATCACCUGC GCCACAGGTG CGGTTGCTGG 1440

CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG 1500

40 CGCTTGTTTC GGCCTGGGTA TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC 1560

45 TCCTTGCATG CACCATTCTT TCGGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC 1620

50 TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CGATGCCCTT GAGAGCCTTC 1680

AACCCAGTCA GCTCCTTCCG GTGGGCGCGG GGCATGACTA TCGTCGCCGC ACTTATGACT 1740

55

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GTCTTCTTTA TCATGCAACT CGTAGGACAG GTGCCGGCAG CGCTCTGGGT CATTTTCGGC 1800

5 GAGGACCGCT TTCGCTGGAG CGCGACGATG ATCGGCCTGT CGCTTGCGGT ATTCGGAATC 1860

10 TTGCACGCCC TCGCTCAAGC CTTCGTCACT GGTCCC GCCA CCAAACGTTT CGGCGAGAAG 1920

CAGGCCATTA TCGCCGGCAT GGCGGCCGAC GCGCTGGGCT ACGTCTTGCT GCGGTTCCGG 1980

15 ACGCGAGGCT GGATGGCCTT CCCCATTATG ATTCTTCTCG CTTCCGGCGG CATCGGGATG 2040

20 CCCGCGTTGC AGGCCATGCT GTCCAGGCAG GTAGATGACG ACCATCAGGG ACAGCTTCAA 2100

25 GGATCGCTCG CGGCTCTTAC CAGCCTAACT TCGATCACTG GACCGCTGAT CGTCACGGCG 2160

ATTTATGCCG CCTCGGCGAG CACATGGAAC GGGTTGGCAT GGATTGTAGG CGCCGCCCTA 2220

30 TACCTTGTCT GCCTCCCCGC GTTGCGTCGC GGTGCATGGA GCCGGGCCAC CTCGACCTGA 2280

35 ATGGAAGCCG GCGGCACCTC GCTAACGGAT TCACCACTCC AAGAATTGGA GCCAATCAAT 2340

TCTTGCGGAG AACTGTGAAT GCGCAAACCA ACCCTTGGA GAACATATCC ATCGCGTCCG 2400

40 CCATCTCCAG CAGCCGCACG CGGCGCATCT CGGGCAGCGT TGGTCTCTGG CCACGGGTGC 2460

45 GCATGATCGT GCTCCTGTCTG TTGAGGACCC GGCTAGGCTG GCGGGGTTGC CTTACTGGTT 2520

50 AGCAGAATGA ATCACCATA CGCGAGCGAA CGTGAAGCGA CTGCTGCTGC AAAACGTCTG 2580

55

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CGACCTGAGC AACAAACATGA ATGGTCTTCG GTTTCCTGT TTCGTAAAGT CTGGAAACGC 2640

5 GGAAGTCAGC GCCCTGCACC ATTATGTTCC GGATCTGCAT CGCAGGATGC TGCTGGCTAC 2700

10 CCTGTGGAAC ACCTACATCT GTATTAACGA AGCGCTGGCA TTGACCCTGA GTGATTTTTC 2760

TCTGGTCCCG CCGCATCCAT ACCGCCAGTT GTTTACCCTC ACAACGTTCC AGTAACCGGG 2820

15 CATGTTCATC ATCAGTAACC CGTATCGTGA GCATCCTCTC TCGTTTCATC GGTATCATTA 2880

20 CCCCCATGAA CAGAAATTCC CCCTTACACG GAGGCATCAA GTGACCAAAC AGGAAAAAAC 2940

CGCCCTTAAC ATGGCCCGCT TTATCAGAAG CCAGACATTA ACGTTTCTGG AGAAACTCAA 3000

25 CGAGCTGGAC GCGGATGAAC AGGCAGACAT CTGTGAATCG CTTACCGACC ACGCTGATGA 3060

30 GCTTTACCGC AGCTGCCTCG CGCGTTTCGG TGATGACGGT GAAAACCTCT GACACATGCA 3120

35 GCTCCCGGAG ACGGTCACAG CTTGTCTGTA AGCGGATGCC GGGAGCAGAC AAGCCCGTCA 3180

GGGCGCGTCA GCGGGTGTG GCGGGTGTG GGGCGCAGCC ATGACCCAGT CACGTAGCGA 3240

40 TAGCGGAGTG TATACTGGCT TAACTATGCG GCATCAGAGC AGATTGTACT GAGAGTGCAC 3300

45 CATATGCGGT GTGAAATACC GCACAGATGC GTAAGGAGAA AATACCGCAT CAGGCGCTCT 3360

TCCGCTTCCT CGCTCACTGA CTCGCTGCGC TCGGTCGTTT GGCTGCGGCG AGCGGTATCA 3420

50 GCTCACTCAA AGGCGGTAAT ACGGTTATCC ACAGAATCAG GGGATAACGC AGGAAAGAAC 3480

55

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ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG AACCGTAAAA AGGCCGCGTT GCTGGCGTTT 3540

5 TTCCATAGGC TCCGCCCCC TGACGAGCAT CACAAAAATC GACGCTCAAG TCAGAGGTGG 3600

10 CGAAACCCGA CAGGACTATA AAGATACCAG GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC 3660

TCTCCTGTTT CGACCCTGCC GCTTACCGGA TACCTGTCCG CTTTCTCCC TTCGGGAAGC 3720

15 GTGGCGCTTT CTCAATGCTC ACGCTGTAGG TATCTCAGTT CGGTGTAGGT CGTTCGCTCC 3780

20 AAGCTGGGCT GTGTGCACGA ACCCCCCGTT CAGCCCGACC GCTGCGCCTT ATCCGGTAAC 3840

TATCGTCTTG AGTCCAACCC GGTAAGACAC GACTTATCGC CACTGGCAGC AGCCACTGGT 3900

25 AACAGGATTA GCAGAGCGAG GTATGTAGGC GGTGCTACAG AGTTCTTGAA GTGGTGGCCT 3960

30 AACTACGGCT AACTAGAAG GACAGTATTT GGTATCTGCG CTCTGCTGAA GCCAGTTACC 4020

35 TTCGAAAAA GAGTTGGTAG CTCTGATCC GGCAAAACAA CCACCGCTGG TAGCGGTGGT 4080

TTTGTGTTT GCAAGCAGCA GATTACGCGC AGAAAAAAG GATCTCAAGA AGATCCTTTG 4140

40 ATCTTTTCTA CGGGTCTGA CGCTCAGTGG AACGAAACT CACGTTAAGG GATTTTGGTC 4200

45 ATGAGATTAT CAAAAGGAT CTTACCTAG ATCCTTTTAA ATTAAAAATG AAGTTTAAA 4260

TCAATCTAAA GTATATATGA GTAACTTGG TCTGACAGT ACCAATGCTT AATCAGTGAG 4320

50

55

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GCACCTATCT CAGCGATCTG TCTATTTCTG TCATCCATAG TTGCCTGACT CCCCCTCGTG 4380

5 TAGATAACTA CGATACGGGA GGGCTTACCA TCTGGCCCCA GTGCTGCAAT GATACCGCGA 4440

10 GACCCACGCT CACCGGCTCC AGATTTATCA GCAATAAACC AGCCAGCCGG AAGGGCCGAG 4500

CGCAGAAGTG GTCCTGCAAC TTTATCCGCC TCCATCCAGT CTATTAATTG TTGCCGGGAA 4560

15 GCTAGAGTAA GTAGTTCGCC AGTTAATAGT TTGCGCAACG TTGTTGCCAT TGCTGCAGGC 4620

20 ATCGTGGTGT CACGCTCGTC GTTTGGTATG GCTTCATTCA GCTCCGGTTC CCAACGATCA 4680

AGGCGAGTTA CATGATCCCC CATGTTGTGC AAAAAAGCGG TTAGCTCCTT CGGTCTCCG 4740

25 ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG TTATCACTCA TGGTTATGGC AGCACTGCAT 4800

30 AATTCTCTTA CTGTCATGCC ATCCGTAAGA TGCTTTTCTG TGA CTGGTGA GTACTCAACC 4860

AAGTCATTCT GAGAATAGTG TATGCGGCGA CCGAGTTGCT CTTGCCCCGC GTCAACACGG 4920

35 GATAATACCG CGCCACATAG CAGAACTTTA AAAGTGCTCA TCATTGGAAA ACGTTCTTCG 4980

40 GGGCGAAAAC TCTCAAGGAT CTTACCGCTG TTGAGATCCA GTTCGATGTA ACCCACTCGT 5040

45 GCACCCAACT GATCTTCAGC ATCTTTTACT TTCACCAGCG TTTCTGGGTG AGCAAAAACA 5100

GGAAGGCAAA ATGCCGCAAA AAAGGGAATA AGGGCGACAC GGAAATGTTG AATACTCATA 5160

50 CTCTTCCTTT TTCAATATTA TTGAAGCATT TATCAGGTT ATTGTCTCAT GAGCGGATAC 5220

55

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ATATTTGAAT GTATTTAGAA AAATAAACAA ATAGGGGTTC CGCGCACATT TCCCCGAAAA 5280

5 GTGCCACCTG ACGTCTAAGA AACCATTTATT ATCATGACAT TAACCTATAA AAATAGGCGT 5340

10 ATCACGAGGC CCTTTCGTCT TCAAGAATTA ATTGTTATCC GTCACAATT AATTCTTGAC 5400

AATTAGTTAA CTATTTGTTA TAATGTATTC ATAAGCTT 5438

15

INFORMATION FOR SEQ ID NO: 26:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

25 (C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

30 GCTGCCGAAC AGAAGTCTAA 20

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INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

45 (ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

50 CTCGAAGGAA CAATGGATAC 20

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55

INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

5

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

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GTACATATTG TCGTTAGAAC GCG

23

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INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

25

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

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(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

TAATACGACT CACTATAGGG AGA

23

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INFORMATION FOR SEQ ID NO: 30:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

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GCGGATCCTG ATGTCTATTT CATCTTCT

28

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INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(ii) MOLECULE TYPE: Other nucleic acid; Synthetic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

ATCTCGAGTT TTATGCTGCT GCGCCAGCGA

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20 Claims

1. A *Chlamydia pneumoniae* antigenic polypeptide, which comprises polypeptide A containing a sequence of at least 5 consecutive amino acids in the polypeptide of SEQ ID NO: 1.
2. The antigenic polypeptide of claim 1, wherein said polypeptide A is a polypeptide in which at least one amino acid is deleted from the polypeptide of SEQ ID NO: 1.
3. The antigenic polypeptide of claim 1, wherein said polypeptide A is a polypeptide in which at least one amino acid in the polypeptide of SEQ ID NO: 1 is replaced with other amino acid or a polypeptide in which at least one amino acid is added in the polypeptide of SEQ ID NO: 1.
4. The antigenic polypeptide of claim 1, wherein said polypeptide A is a polypeptide in which an amino acid or a peptide sequence is bound to a sequence of at least 5 consecutive amino acids in the polypeptide of SEQ ID NO: 1.
5. The antigenic polypeptide of claim 1, wherein said polypeptide A is a polypeptide containing the amino acid sequence of SEQ ID NO: 1.
6. The antigenic polypeptide of claim 1, wherein said polypeptide A is a polypeptide containing the amino acid sequence of SEQ ID NO: 2.
7. The antigenic polypeptide of claim 1, wherein said polypeptide A is a polypeptide containing the amino acid sequence of SEQ ID NO: 5.
8. A DNA encoding the antigenic polypeptide of any one of claims 1-7, or a DNA complementary thereto.
9. The DNA of claim 8, which contains the base sequence of SEQ ID NO: 3.
10. The DNA of claim 8, which contains the base sequence of SEQ ID NO: 4.
11. The DNA of claim 8, which contains the base sequence of SEQ ID NO: 7.
12. A recombinant vector carrying the DNA of any one of claims 8-11.
13. The recombinant vector of claim 12, which is plasmid pCPN533 α containing the base sequence of SEQ ID NO: 10.
14. A transformant containing the recombinant vector of claim 12 or 13.
15. A method for production of an anti-*Chlamydia pneumoniae* antibody, wherein the antigenic polypeptide of any one of claims 1-7 is used as an antigen.

16. A method for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, wherein the antigenic polypeptide of any one of claims 1-7 is used as an antigen.
- 5 17. A reagent for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, which comprises the antigenic polypeptide of any one of claims 1-7 as an antigen.
18. A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the antigenic polypeptide of any one of claims 1-7 as an active ingredient.
- 10 19. A fused protein of a Chlamydia pneumoniae antigenic polypeptide with dihydrofolate reductase, in which polypeptide B containing a sequence of at least 5 consecutive amino acids in the polypeptide of SEQ ID NO: 1 is bound to the polypeptide of SEQ ID NO: 14 either directly or via an intervening amino acid or amino acid sequence.
- 15 20. The fused protein of claim 19, wherein said polypeptide B is a polypeptide in which at least one amino acid is deleted from the polypeptide of SEQ ID NO: 1.
21. The fused protein of claim 19, wherein said polypeptide B is a polypeptide in which at least one amino acid in the polypeptide of SEQ ID NO: 1 is replaced with other amino acids or a polypeptide in which at least one amino acid is added in the polypeptide of SEQ ID NO: 1.
- 20 22. The fused protein of claim 19, which is a polypeptide containing the amino acid sequence of SEQ ID NO: 15.
23. The fused protein of claim 19, which is a polypeptide containing the amino acid sequence of SEQ ID NO: 16.
- 25 24. A DNA encoding the fused protein of any one of claims 19-23, or a DNA complementary thereto.
25. The DNA of claim 24, which contains the base sequence of SEQ ID NO: 17.
26. The DNA of claim 24, which contains the base sequence of SEQ ID NO: 18.
- 30 27. A recombinant vector carrying the DNA of any one of claims 24-26.
28. The recombinant vector of claim 27, which is plasmid pCPN533T.
- 35 29. A transformant containing the recombinant vector of claim 27 or 28.
30. A method for production of an anti-Chlamydia pneumoniae antibody, wherein the fused protein of any one of claims 19-23 is used as an antigen.
- 40 31. A method for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, wherein the fused protein of any one of claims 19-23 is used as an antigen.
32. A reagent for detection and/or measurement of an anti-Chlamydia pneumoniae antibody, which comprises the fused protein of any one of claims 19-23 as an antigen.
- 45 33. A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the fused protein of any one of claims 19-23 as an active ingredient.
34. A probe for detection and/or measurement of Chlamydia pneumoniae gene, which comprises any one of
 - (a) a DNA containing a sequence of at least 10 consecutive bases in the DNA of SEQ ID NO: 3,
 - (b) a DNA complementary to DNA (a), or
 - (c) a DNA having at least 90% homology to DNA (a) or (b).
- 50 35. The probe of claim 34, which contains the base sequence of SEQ ID NO: 19.
36. The probe of claim 34, which contains the base sequence of SEQ ID NO: 20.
37. A method for detection and/or measurement of Chlamydia pneumoniae gene, wherein the probe of any one of

claims 34-36 is used.

38. A reagent for detection and/or measurement of Chlamydia pneumoniae gene, which comprises the probe of any one of claims 34-36.

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39. A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the probe of any one of claims 34-36 as an active ingredient.

40. A primer for detection and/or measurement of Chlamydia pneumoniae gene, which comprises any one of

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- (a) a DNA containing a sequence of at least 10 consecutive bases in the DNA of SEQ ID NO: 3,
- (b) a DNA complementary to DNA (a), or
- (c) a DNA having at least 90% homology to DNA (a) or (b).

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41. The primer of claim 40, which contains the base sequence of SEQ ID NO: 19.

42. The primer of claim 40, which contains the base sequence of SEQ ID NO: 20.

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43. A method for detection and/or measurement of Chlamydia pneumoniae gene, wherein the primer of any one of claims 40-42 is used.

44. A reagent for detection and/or measurement of Chlamydia pneumoniae gene, which comprises the primer of any one of claims 40-42.

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45. A reagent for diagnosis of a Chlamydia pneumoniae infection, which comprises the primer of any one of claims 40-42 as an active ingredient.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP95/01896

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl ⁶ C07K14/295, C12N15/31, C12N1/21, C12P21/02, C12P21/08, C12Q1/68, G01N33/569 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. Cl ⁶ C07K14/295, C12N15/31, C12N1/21, C12P21/02, C12P21/08, C12Q1/68, G01N33/569 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAS ONLINE, WPI, WPI/L, BIOSIS PREVIEWS		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIKUTA L. C. et al., "Isolation and Sequence Analysis of the Chlamydia pneumoniae GroE Operon" INFECTION AND IMMUNITY, Dec. 1991, Vol. 59, No. 12, pages 4665-4669	1 - 15, 19 - 30
A	KORNAK J. M. et al., "Sequence Analysis of the Gene Encoding the Chlamydia pneumoniae DnaK Protein Homolog" INFECTION AND IMMUNITY, Feb. 1991, Vol. 59, No. 2, pages 721-725	1 - 14, 19 - 29
A	MELGOSA M. P. et al., "Sequence Analysis of the Major Outer membrane Protein Gene of Chlamydia pneumoniae" INFECTION AND IMMUNITY, Jun. 1991, Vol. 59, No. 6, pages 2195-2199	1 - 14, 19 - 29
A	JP, 4-297871, A (Hitachi Chemical Co., Ltd.), October 21, 1992 (21. 10. 92) & EP, 456524, A1 & US, 5318892, A	16 - 18, 31 - 33
A	JP, 5-317097, A (Fuso Pharmaceutical Co., Ltd.),	34 - 45
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "T" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search December 8, 1995 (08. 12. 95)		Date of mailing of the international search report December 26, 1995 (26. 12. 95)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.		Authorized officer Telephone No.

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EP 0 784 059 A1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP95/01896

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	December 3, 1993 (03. 12. 93) & EP, 402993, A1 & CA, 2017520, A & FI, 9002990, A & US, 5085986, A & KR, 9209424, B1	

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